

CHAPTER 4

RESULTS

4.3 Laboratory studies

The aim of the laboratory work was to compare the growth of *Chlorella* in the presence and absence of supplemented CO₂. There are two sets of laboratory experiments.

4.3.1: Experiment I - Effect of CO₂ supplementation at 15 min h⁻¹ in RE and BBM (Table 4.3) [Figure 4.2 and 4.3]

Growth of *Chlorella vulgaris* was highest in RE supplemented with molasses and CO₂ compared to all the other substrates. Specific growth rate of *Chlorella vulgaris* in RE + CO₂, $\mu=0.64 \text{ day}^{-1}$ was much higher than RE + air, $\mu=0.57 \text{ day}^{-1}$. It is interesting to note that RE + CO₂ + MOL had a much higher growth rate than that of RE + CO₂:- $\mu_{\text{RE} + \text{MOL}} = 0.74$, $\mu_{\text{RE} + \text{CO}_2 + \text{MOL}} = 0.81$.

4.3.2: Experiment II - Effect of CO₂ supplementation at 25min h⁻¹ (Table 4.4) [Figure 4.4 to 4.6]

Increasing the CO₂ aeration time to 25min.h⁻¹ improved the growth, ($\mu_{\text{RE}+\text{CO}_2} = 0.56\text{day}^{-1}$) compared to $\mu_{\text{RE}+\text{MOLL}} = 0.45\text{day}^{-1}$) indicating that that the cultures could be CO₂ limited. Biomass in terms of Chll-a was also highest in the RE + CO₂ treatment.

The Chll:DW ratio were similar for the RE, RE+AIR and RE+CO₂ treatments. It was low when molasses was added. The Chll:cell ratio however was much higher in BBM and RE

TABLE 4.3 : Exp. I – Growth of *Chlorella vulgaris* in 1L flasks aerated with 5% CO₂ at 1L min⁻¹, 15min hr⁻¹ – Day 4 data

| GROWTH MEDIUM | (BBM) | BBM + CO ₂ | RE + AIR | RE + CO ₂ | RE + MOL | RE + MOL + CO ₂ |
|--------------------------------------|-------|-----------------------|----------|----------------------|----------|----------------------------|
| Parameters | | | | | | |
| Cell Count (cells ml ⁻¹) | 5.99 | 4.97 | 10.03 | 13.00 | 19.70 | 25.75 |
| μ (day ⁻¹) | 0.44 | 0.40 | 0.57 | 0.64 | 0.74 | 0.81 |
| pH | 6.9 | 6.5 | 8.0 | 7.6 | 6.9 | 6.7 |

BBM : Bold Basal Medium, Control

CO₂ : 5% Carbon Dioxide

RE : Rubber Effluent

MOL : 0.05% molasses

Samples taken at Day 4 of growth

TABLE 4.4 : Exp. II – Growth of *Chlorella vulgaris* in 1L flasks aerated with 5% CO₂ at 1L min⁻¹, 25min hr⁻¹ – Day 5 data

| GROWTH MEDIUM | BBM | RE | RE + AIR | RE + CO ₂ | RE + MOL | RE + MOL + CO ₂ |
|--------------------------------------|------|-------|----------|----------------------|----------|----------------------------|
| Parameters | | | | | | |
| Cell Count (cells ml ⁻¹) | 5.05 | 13.87 | 14.49 | 36.63 | 21.75 | 14.71 |
| μ (day ⁻¹) | 0.16 | 0.36 | 0.37 | 0.56 | 0.45 | 0.37 |
| Chl-a (mg m ⁻³) | 3.79 | 6.24 | 3.78 | 6.39 | 4.41 | 2.56 |
| DW (mgL ⁻¹) | 86.7 | 102.0 | 65.8 | 121.0 | 173.0 | 235.5 |
| pH | 7.31 | 7.88 | 7.98 | 6.95 | 8.19 | 6.84 |
| Chl : DW (X10 ²) | 4.37 | 6.12 | 5.74 | 5.28 | 2.55 | 1.09 |
| chl : cell (X10 ⁻⁸) | 74.9 | 44.9 | 26.1 | 17.4 | 20.2 | 17.4 |

BBM : Bold Basal Medium, Control

CO₂ : 5% Carbon Dioxide

RE : Rubber Effluent

MOL : 0.05% molasses

Samples taken at Day 5 of growth

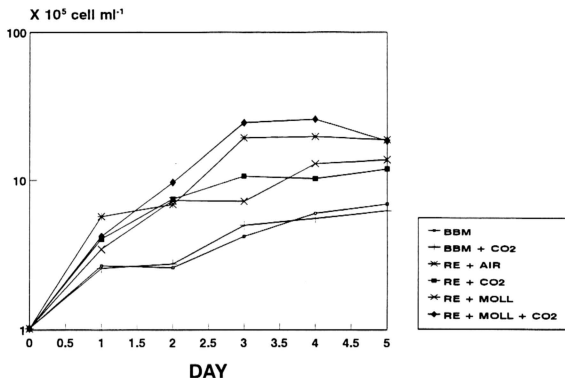


FIG 4.2 : EXPERIMENT I - Semilogarithmic plots of growth curve of *Chlorella vulgaris* grown in BBM and RE supplemented with CO₂ and molasses.

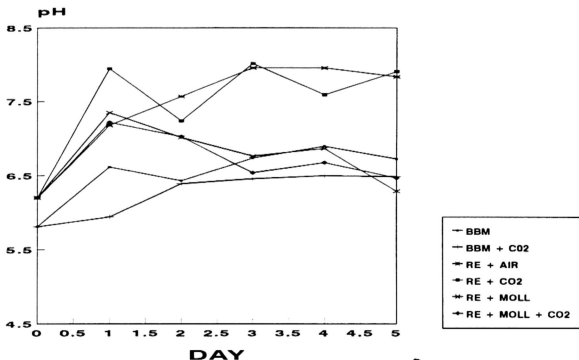


FIG 4.3 : EXPERIMENT I - Daily pH changes in flask cultures of *Chlorella vulgaris* in BBM & RE supplemented with CO₂ and molasses

alone. This is probably due to the lower growth rates in BBM and RE.

4.3.3 pH and other variations during the growth of algae in the flasks

Final pH values were generally higher in flasks aerated with air only compared to that aerated with CO₂. Figure 4.3 and Figure 4.6 show the pH trends in the various flasks. In most cases the pH increased and stabilized after day 2. The most significant increase was in the flask containing RE aerated with air, while in the flask aerated with CO₂ the pH remained stable (Figure 4.6).

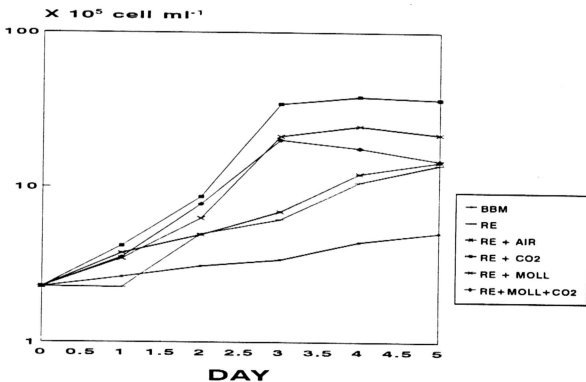


FIG 4.4 : EXPERIMENT II - Semilogarithmic plot for growth of *Chlorella vulgaris* grown in BBM and RE supplemented with CO₂ and molasses

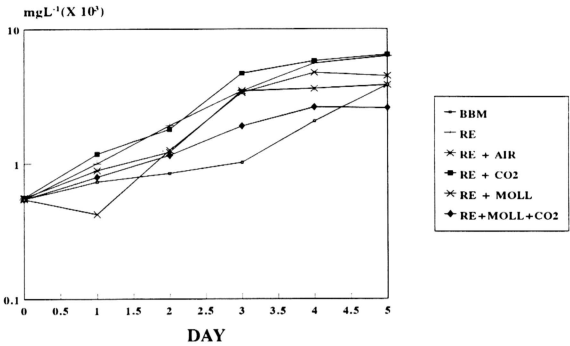


FIG 4.5 : EXPERIMENT II - Semilogarithmic plot of chlorophyll-a values of *Chlorella vulgaris* grown in BBM and RE supplemented with CO₂ and molasses

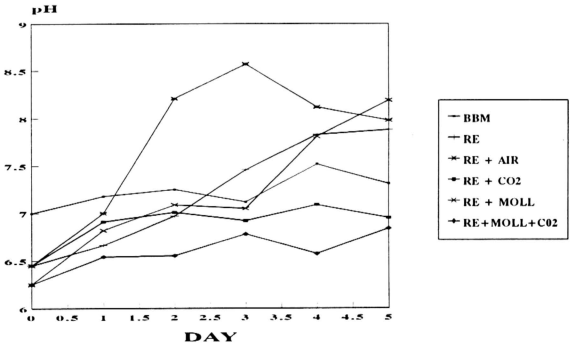


FIG 4.6 : EXPERIMENT II - Daily pH in flask cultures of *Chlorella vulgaris* grown in BBM and RE supplemented with CO₂ and molasses

4.4: Growth and Biomass Production of *Chlorella* in the High Rate Algal Pond

4.4.1: Growth curves based on cell count and chl-a

In general *Chlorella* growth in all the HRAP batches followed a sigmoidal growth curve (Figure 4.7 to Figure 4.11). In all batches, supplementation with CO₂ or molasses or both enhanced algal concentration. Growth curves based on cell count and chl-a correlate well (Figures 4.7 to 4.11).

Batch I {Figure 4.7}

Cell concentration of *Chlorella* was higher in the CO₂ supplemented pond than the control. Algal growth peaked at day 2 and plateaued out after that. There was greater decrease in *Chlorella* cell number in the control pond after biomass peaked.

Batch II {Figure 4.8}

Cell concentration and chlorophyll content was again higher in the pond aerated with CO₂

Batch III {Figure 4.9}

In this batch CO₂ aeration rate was increased to 40min.h⁻¹ as compared to 20min.h⁻¹ in the previous batches. However growth in the pond supplemented with CO₂ did not increase significantly higher than the control pond.

Batch IV {Figure 4.10}

The purpose of this batch is to compare organic carbon(molasses) supplementaion to inorganic carbon(CO₂)

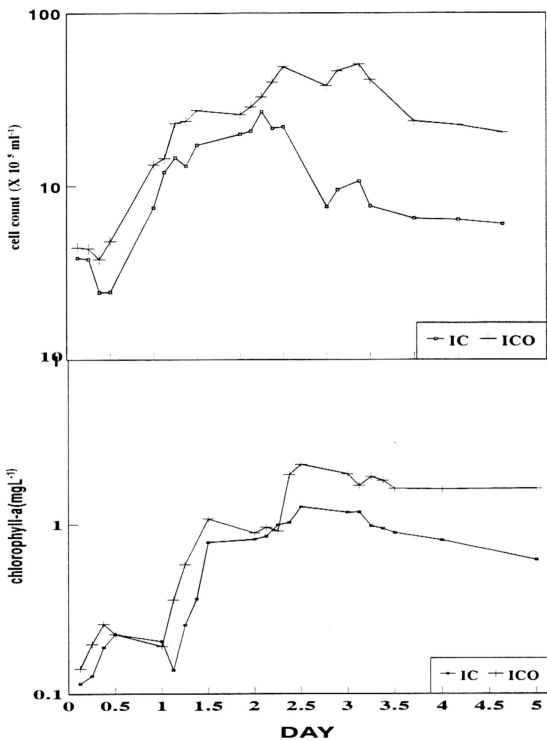


Fig 4.7: BATCH I - Semilogarithmic plot of chl-a and cell count for *Chlorella vulgaris* grown in HRAP where 5% CO₂ in air was bubbled into pond ICO from 0630 to 1830h at 20min h⁻¹ daily at flowrate of 5Lmin⁻¹

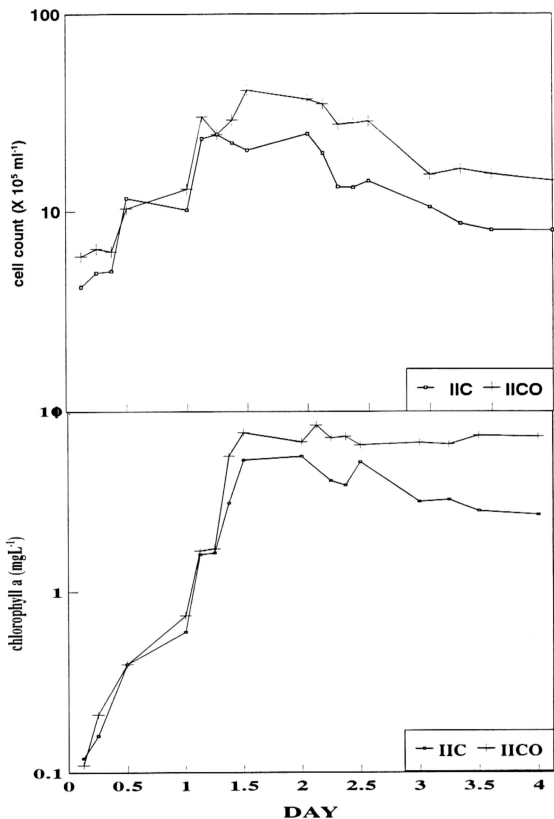


FIG 4.8: BATCH II Semilogarithmic plot of Chl-a and cell count for *Chlorella vulgaris* grown in HRAP where 5% CO_2 was bubbled into pond IICO from 0630 1830 at 20 min h^{-1} daily at flowrate at 5 L min^{-1}

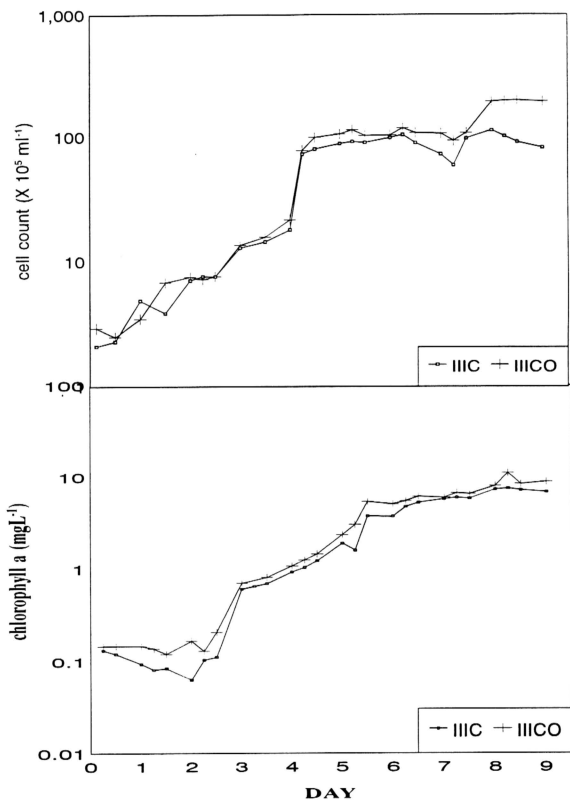


FIG 4.9: BATCH III - Semilogarithmic plot Chll-a and cell count for *Chlorella vulgaris* grown in HRAP where 5% CO₂ was bubbled into pond IIICO from 0630 to 1830h at 40min h⁻¹ daily at flowrate at 5L min⁻¹

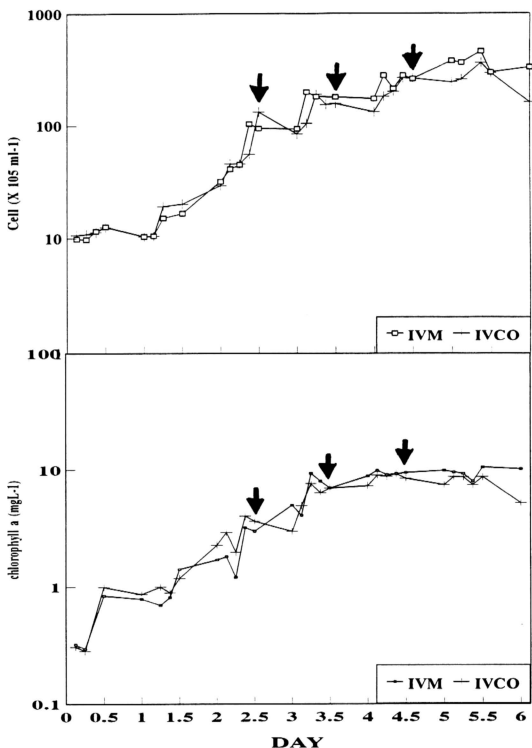


FIG 4.10: BATCH IV - Semilogarithmic plot of Chl-a and cell forh *Chlorella vulgaris* grown in HRAP grown in HRAP where 5% CO₂ in air was bubbled into pond IVCO from 0630 to 1830h at 40min h⁻¹ at flowrate of 5Lmin⁻¹ and mol. added on day 2,3 & 4 at 1830 into IVM(→)

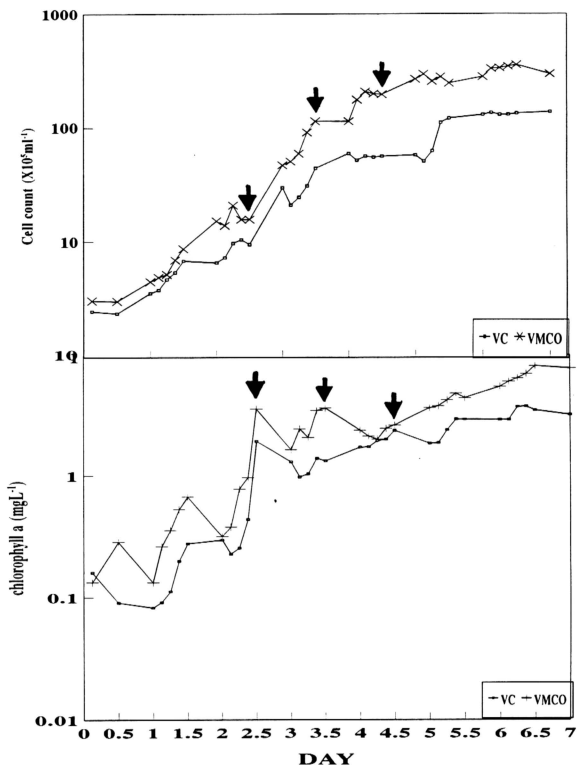


FIG 4.11 BATCH V Semilogarithmic plot of Chl-a and cell count for *Chlorella vulgaris* in HRAP where 5% CO₂ in air was bubbled into VMCO from 0630 to 1830h at 40minh⁻¹ at 40minh⁻¹ and 0.05% molasses was added into VMCO at 1830 on day 2, 3 & 4 (↓)

supplementation. Both growth curves were similar indicating that *Chlorella* was able to utilize both carbon sources efficiently.

Batch V {Figure 4.11}

The pond supplemented with CO₂ and molasses had much higher cell concentration and chlorophyll concentration than the control pond (RE alone).

4.4.2: Specific Growth Rate and Biomass Production

(Table 4.5 and Table 4.6)

RE quality has significant effect on *Chlorella* growth in the HRAP. Based on cell count and chl-a content the RE supplemented with CO₂ and molasses (Batch VMCO) produced the highest in all over the control (RE alone). However the μ was higher in Batches I and II, the RE in these batches had lower levels of TSS and COD, and the algae grew rapidly soon after inoculation. μ in RE alone (control) ranged from 0.94 to 3.50 day⁻¹ based on cell count and from 1.15 to 1.92 based on chlorophyll-a content. Growth in CO₂ supplemented ponds was always higher than the control. The μ of CO₂ supplemented ponds (ICO, IICO, IIICO and IVCO) ranged from 1.01 to 3.58 based on cell count and from 0.96 to 2.94 based on chlorophyll content. Addition of molasses at 18:30h improved algal growth in IVM ($\mu=0.895$) and VM ($\mu=1.41$).

TABLE 4.5: Summary of growth and biomass results in High Rate Algal Pond studies.

| Batch | Day of maximum cell expression | u (day ⁻¹) cell count | u (day ⁻¹) chl - a | Cell Count (X 10 ⁵ cell ml ⁻¹) | | Chl - a (X 10 ³ mg L ⁻¹) | | Algal Biomass (g L ⁻¹) | | Chl : Cell (X 10 ⁻⁸) | |
|--------|--------------------------------|--------------------------------------|-----------------------------------|--|--------|--|-------|---------------------------------------|---------|----------------------------------|--------|
| | | | | Final | Max. | Final | Max. | Final | Max. | Final | Max. |
| I C | 2 | 1.35 | 1.92 | 6.4 | 26.6 | 810 | 1270 | 54.27 | 85.09 | 126.56 | 47.74 |
| I CO | 2 | 1.66 | 2.04 | 22.3 | 48.4 | 1620 | 2270 | 108.54 | 152.09 | 72.65 | 46.90 |
| II C | 2 | 3.51 | 1.6 | 8.05 | 24.7 | 2680 | 5620 | 179.56 | 376.54 | 332.92 | 227.53 |
| II CO | 2 | 3.58 | 2.94 | 14.3 | 37.1 | 7210 | 7580 | 483.07 | 507.86 | 504.20 | 204.31 |
| III C | 4 | 1.23 | 1.8 | 82.4 | 113.3 | 6930 | 6930 | 464.31 | 464.31 | 84.10 | 61.17 |
| III CO | 4 | 1.31 | 2.17 | 195.2 | 200 | 8940 | 11100 | 598.98 | 743.7 | 45.80 | 55.50 |
| IV M | 2 | 0.94 | 0.895 | 335 | 462.67 | 10190 | 10530 | 682.73 | 705.51 | 30.42 | 22.76 |
| IV CO | 2 | 1.01 | 0.964 | 163.5 | 366 | 5240 | 8995 | 351.08 | 602.665 | 32.05 | 24.58 |
| V C | 4 | 0.94 | 1.149 | 138.3 | 138.3 | 3180 | 3720 | 213.06 | 249.24 | 22.99 | 26.90 |
| V MCO | 4 | 1.41 | 1.19 | 297 | 356 | 7740 | 8090 | 518.58 | 542.03 | 26.06 | 22.72 |

NOTE :

Batch I and II : Effluent from Gombak Factory

Batch III, IV and V : Effluent from Atherton Factory

Final = Concentration on the last day of the HIRAP Study

Max. = Maximum concentration obtained during the study

Table 4.6: Comparison of algal biomass concentration in different HRAP batches

| Batch | Supplementation | Cell count | | ChlI-a (mgL^{-1}) | |
|-------|--|------------|-------|------------------------------|-------|
| | | Max. | Final | Max. | Final |
| I | CO ₂ bubbled at 20min hr ⁻¹ | 2.1 | 3.5 | 1.7 | 2.0 |
| II | CO ₂ bubbled at 20min hr ⁻¹ | 1.5 | 1.8 | 1.4 | 2.7 |
| III | CO ₂ bubbled at 40min hr ⁻¹ | 1.8 | 2.4 | 1.6 | 1.3 |
| IV | 0.05% molasses in pond IVM on day 2, 3 and 4, CO ₂ bubbled at 40min hr ⁻¹ in Pond IVCO | 0.8 | 0.5 | 0.9 | 0.5 |
| V | Pond VMCO supplemented with 0.05% mol and CO ₂ bubbled at 40min hr ⁻¹ | 2.6 | 2.2 | 2.2 | 2.4 |

Figures shown are ratios of treatment pond values to control pond values

In Batch IV the ratio is that of CO₂ to molasses

In the above studies CO₂ was bubbled for 12 hours during day time

Algal growth peaked from day 1.5 to day 4.5 . In Batch I algal growth peaked at day 2.5; in Batch II at day 1.5; in Batch III at day 4.0; in Batch IV peaked at day 3.5 and in Batch V peaked at day 4.5 . Maximum algal concentration based on cell count ranged between 25 to 463mgL^{-1} while maximum algal concentration based on chlorophyll ranged from 1270mgL^{-1} to 11100mgL^{-1} .

In Table 4.6 shows the comparison of algal biomass between the control and the treatment ponds. Based on cell count, in all ponds where the control ponds only contained RE, algal concentration was always higher in the treatment pond. The ratio of algal concentration in treatment pond to control pond ranged from 1.5 to 2.6 based on maximum cell count, and from 0.5 to 3.5 based on final cell count; from 0.9 to 2.2 for maximum chl-a content and from 0.5 to 2.7 on final cell count.

Comparing Batches III, IV and V the pond VMCO supplemented with both CO_2 and molasses produced good growth with ratios of up to 2.6 .

4.4.3: Autotrophic and heterotrophic growth (Table 4.7)

In Table 4.7 autotrophic growth was highest during the logarithmic phase for all five batches. Heterotrophic growth was highest in Pond IVM where molasses was added to RE ($\mu=0.06\text{d}^{-1}$). Autotrophic growth was generally higher in ponds supplemented with CO_2 .

Table 4.7 : Specific Autotrophic and Heterotrophic Growth Rate of *Chlorella vulgaris* in the HRAP

| DAY | | BATCH I | | BATCH II | | BATCH III | | BATCH IV | | BATCH V | |
|-----|--------|-----------------|-----------------|-----------------|------------------|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|
| | | C | CO | C | CO | C | CO | M | CO | C | MCO |
| 0 | A H | -0.04 0.1 | 0.005 0.08 | 0.51 -0.011 | 0.046 0.019 | 0.008 0.06 | 0.013 0.027 | 0.013 0.009 | 0.098 -0.016 | 0.001 0.028 | 0.015 0.032 |
| 1 | A H | 0.07 0.01 | 0.059 -0.004 | 0.058 0.016 | 0.096 -0.009 | -0.019 0.05 | 0.056 0.008 | 0.04 0.055 | 0.056 0.032 | 0.054 -0.003 | 0.055 0.047 |
| 2 | A H | 0.008 -0.08 | 0.053 -0.021 | 0.046 -0.025 | -0.021 -0.052 | 0.006 0.044 | 0.001 0.048 | 0.09 -0.001 | 0.125 -0.028 | 0.03 0.096 | 0.026 0.047 |
| 3 | A H | 0.018 0.014 | 0.007 -0.046 | -0.02 0 | 0.001 -0.006 | 0.009 0.019 | 0.012 0.027 | 0.027 0.024 | 0.042 -0.013 | 0.033 0.01 | 0.073 0 |
| 4 | A H | 0.006 -0.006 | 0.004 -0.009 | | | 0.143 -0.01 | 0.127 0.005 | 0.025 0.04 | 0.057 -0.006 | 0.0103 0.022 | 0.045 0.025 |
| 5 | A H | | | | | 0.015 0.008 | 0.007 -0.008 | 0.016 0.009 | 0.015 -0.049 | 0.061 0.006 | 0.004 0 |
| 6 | A H | | | | | 0.004 -0.029 | 0.003 0.0009 | | | 0.002 0.003 | 0.202 -0.015 |
| 7 | A H | | | | | 0.024 0.012 | 0.096 0.049 | | | | |
| 8 | A H | | | | | 0.017 -0.008 | 0.002 -0.002 | | | | |

A = autotrophic growth (hr^{-1})
H = heterotrophic growth (hr^{-1})

4.5 POLLUTION PARAMETERS

Table 4.8 summarizes the treatment efficiencies of each of the HRAP batches. Best reductions in pollution parameters was obtained for COD values followed by ammoniacal nitrogen and dissolved orthophosphate.

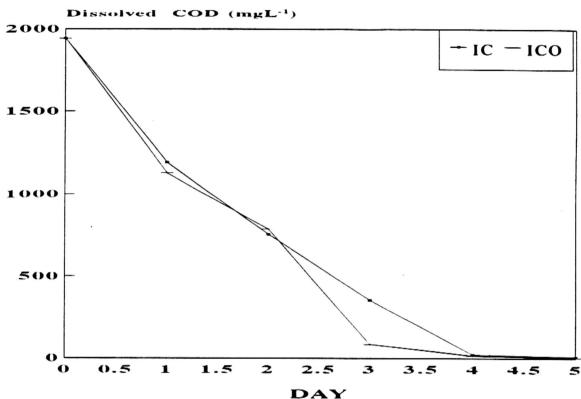


FIG 4.12: Chemical Oxygen Demand in HRAP (Batch I) where 5% CO₂ in air was bubbled in daily from 6:30 to 18:30

All five HRAP batches generally showed a good reduction in COD levels (93.3 - 99.7%) except for VMCO (55.8%) and IVM (80.7%). In Batches I, II and III (Fig 4.12 to Fig 4.14) COD levels decreased with time. Addition of mollasses seemed to increase the COD when first added

Table 4.8 : Summary of reduction in pollution parameters of RE monitored during HRAP batches

| Pond | C.O.D. (mgL ⁻¹) | | | NH ₄ -N (mgL ⁻¹) | | | PO ₄ -P (mgL ⁻¹) | | |
|---------------|-----------------------------|----------|--------|---|----------|--------|---|----------|--------|
| | Influent | Effluent | % Red. | Influent | Effluent | % Red. | Influent | Effluent | % Red. |
| IC ICO | 1943 | 10 | 99.5% | 124.70 | 75.30 | 39.6% | 10.04 | 7.81 | 22.2% |
| | 1942 | 5 | 99.7% | 172.97 | 29.65 | 82.9% | 13.71 | 6.51 | 52.5% |
| IIC IICO | 1270 | 84 | 93.4% | 174.39 | 73.37 | 57.9% | 15.12 | 8.057 | 46.7% |
| | 1270 | 70 | 94.5% | 174.39 | 77.50 | 55.6% | 15.12 | 7.82 | 48.3% |
| IIIC IIICO | 4195 | 224 | 94.7% | 407.07 | 21.70 | 94.7% | 55.23 | 29.82 | 46.0% |
| | 4195 | 140 | 96.7% | 407.75 | 40.80 | 90.0% | 62.29 | 44.9 | 27.9% |
| IVC IVCO | 2443 | 472 | 80.7% | 283.80 | 18.07 | 93.6% | 22.70 | 18.57 | 18.2% |
| | 2432 | 52 | 97.9% | 299.69 | 40.01 | 86.6% | 22.53 | 20.22 | 10.3% |
| VC VCO | 2610 | 106 | 95.9% | 367.73 | 174.93 | 52.4% | 25.68 | 20.47 | 20.3% |
| | 2470 | 1092 | 55.8% | 408.00 | 144.76 | 64.5% | 30.25 | 22.09 | 27.0% |

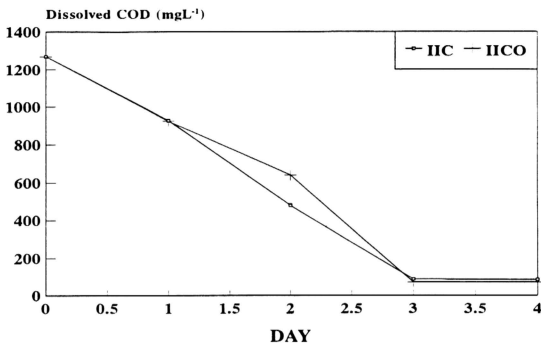


FIG 4.13: Chemical oxygen demand in HRAP (Batch II) where 5% CO₂ was bubbled in daily from 6:30 to 18:30

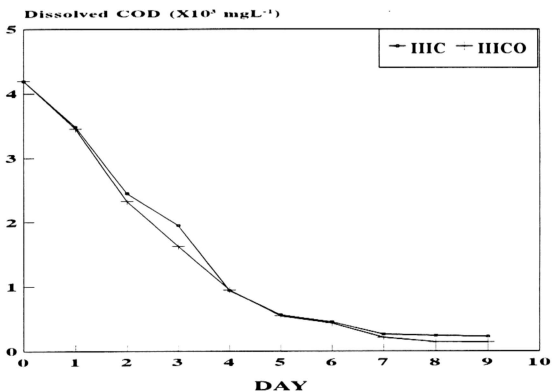


FIG 4.14 : Chemical Oxygen Demand in RE treated in a HRAP system (Batch III) where 5% CO₂ is bubbled in daily from 6:30 to 18:30

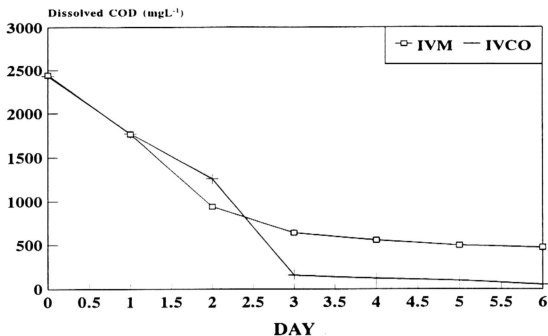


FIG 4.15 : Chemical Oxygen Demand in RE treated in a HRAP system (Batch IV) where 5% CO₂ was bubbled in daily in IVCO from 06:30 to 18:30 and 0.05% molasses was added in IVM on day 2, 3 & 4 at 18:30

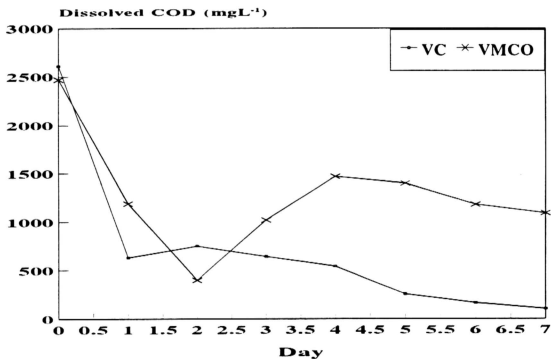


FIG 4.16 : Chemical Oxygen Demand in RE of HRAP (Batch V) where 5% CO₂ was bubbled into pond 2 daily from 6:30 to 18:30 and 0.05% molasses was added in on day 2,3 & 4 at 18:30

into the pond (Ponds IVM and VMCO - Figure 4.15 and 4.16). Generally algal biomass helped reduce the COD in the effluent as observed in Batches I, II and III when CO₂ supplementation increased algal growth and gave better reduction than the control (IC, IIC and IIIC) ponds. For the IVM and VMCO ponds the presence of recalcitrant constituents in the molasses (dark brown substances) contributed to lower COD reduction. The generally better COD reduction obtained compared to NH₄N and PO₄ show carbon limitation in the RE.

The dissolved COD values reduced rapidly from day 0 (Fig 4.12 to Fig 4.16). The filtered COD content in the final effluent (ponds without molasses) ranged from 5 - 52 mgL⁻¹. Dissolved COD values in ponds IVM and VMC were 472 mgL⁻¹ and 1092 mgL⁻¹ respectively. The final effluent from these pond had a brownish tinge while those from the other ponds were clear. The best reduction (99.5% and 99.9%) was observed in Ponds IC and ICO where the initial value of the dissolved COD is the lowest (1943 mg/l). There was no significant difference between COD values of ponds supplemented with CO₂ and those not supplemented with CO₂.

Ponds IVM and VMC showed corresponding increase in COD values in day, 2, 3 and 4 when molasses was added. However after day 4 a decrease in COD values was observed indicating that the algal biomass was effective in COD reduction.

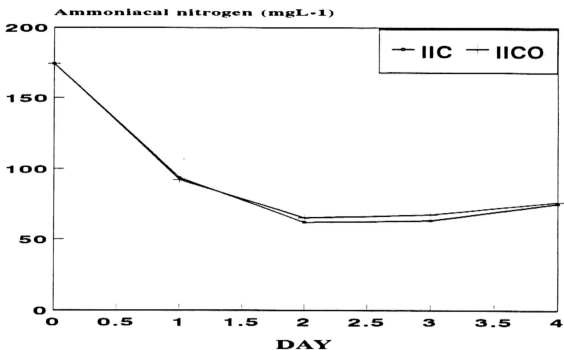


FIG 4.18 : Ammoniacal nitrogen in RE of HRAP (Batch II) where 5% CO₂ was bubbled in daily from 6:30 to 18:30

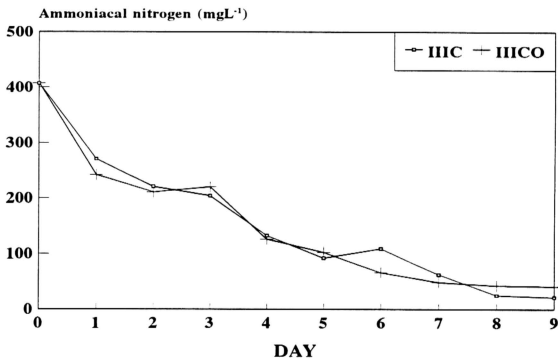


FIG 4.19 : Ammoniacal nitrogen in RE of HRAP (Batch III) where 5% CO₂ was bubbled in daily in IIICO from 6:30 to 18:30

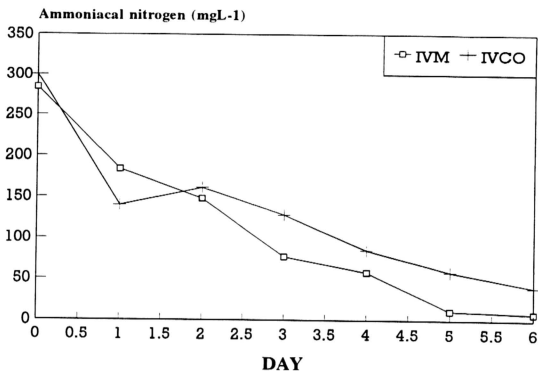


FIG 4.20 : Ammoniacal nitrogen in RE of HRAP (Batch IV) where 5% CO₂ was bubbled in daily from 6:30 to 18:30

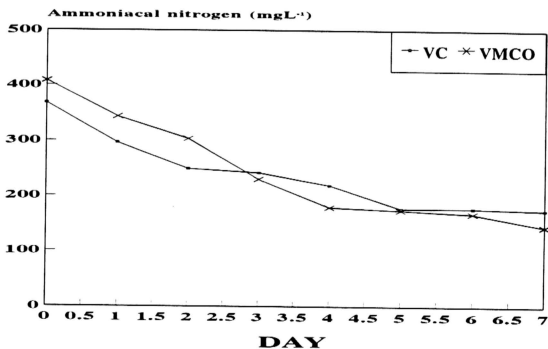


FIG 4.21 : Ammoniacal nitrogen in RE of HRAP (Batch V) where 5% CO₂ was bubbled in daily from 6:30 to 18:30 into pond 2 and 0.05% molasses was added on day 2,3 & 4 at 18:30 in pond 2

4.5.3 Dissolved orthophosphate

The presence of phosphate is vital for algal growth. Reduction in dissolved orthophosphate in the HRAP ranged from 10.3% to 52.5%. The final concentration of phosphate in the filtered effluent ranged from 7.81 mgL^{-1} to 44.9 mgL^{-1} . The ponds treated with molasses (Ponds IVM and VMCO) had higher reductions of 93.6% and 64.5% respectively, which were higher than the control pond.

In all the HRAP batches dissolved orthophosphate reduced steadily with increase in algal biomass and stabilized during the stationary phase.[Fig. 4.22 to Fig 4.26]

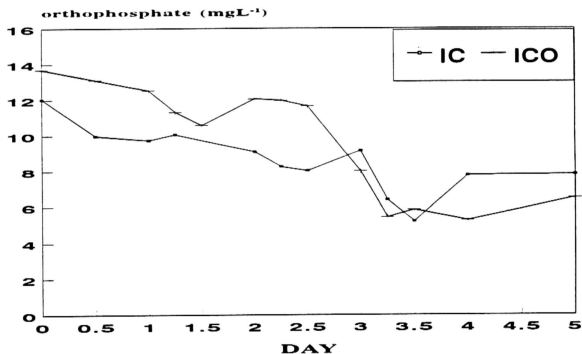


FIG 4.22 : Orthophosphate changes in RE of HRAP (Batch I) where 5% CO_2 was bubbled in daily from 6:30 to 18:30

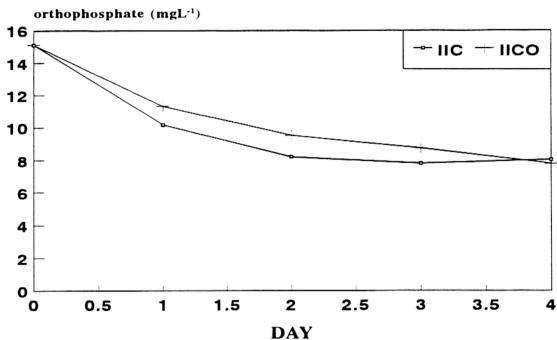


FIG 4.23 : Orthophosphate changes in RE of HRAP (Batch II) where 5% CO_2 was bubbled in daily from 6:30 to 18:30

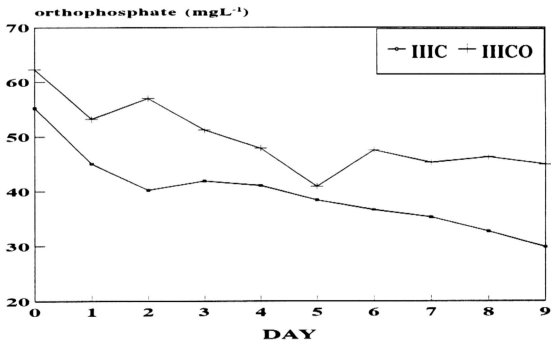


FIG 4.24 : Orthophosphate changes in RE of HRAP (Batch III) where 5% CO_2 was bubbled in daily from 6:30 to 18:30

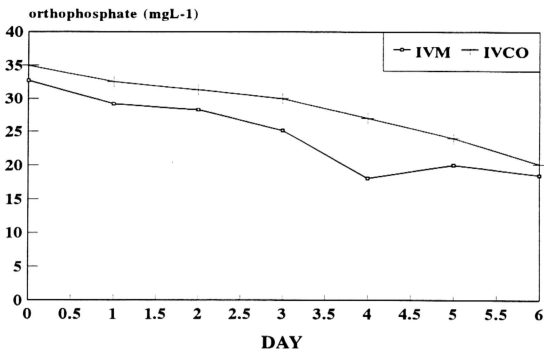


FIG 4.25 : Orthophosphate changes in RE of HRAP (Batch IV) where 5% CO₂ was bubbled in daily from 06:30 to 18:30h in IVCO and 0.05% molasses was added in IVM on day 2,3 & 4 at 18:30h

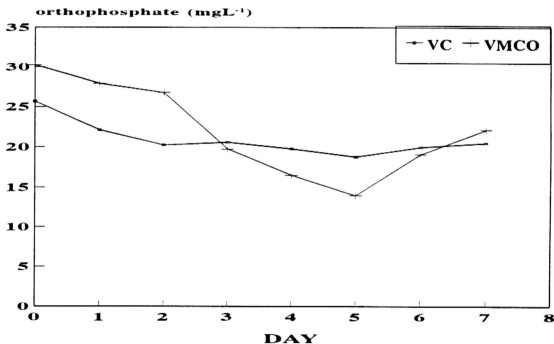


FIG 4.26: Orthophosphate changes in the HRAP (Batch V) where 5% CO₂ was bubbled in pond 2 from 6:30 to 18:30 and 0.05% molasses was also added in pond 2 on day 2,3 and 4 at 18:30

4.5.4 Total solids (TS) and Total suspended solids (TSS)

Figures 4.27 to 4.31 shows the TS values during the batches runs while Figures 4.32 to 4.36 show TSS trends. Supplementaion with molasses produced much higher TS and TSS than the controls. The higher values are attributed to bacterial biomass which proliferate on the molasses.

4.6: PHYSICAL PARAMETERS IN THE HRP

The physical parameters monitored were dissolved oxygen, pH, temperature and light. Table 4.9 summarizes the range of physical parameters observed during the study period. Daily, three measurements were taken, that is at 0630, 1230 and 1830h.

TABLE 4.9: Range of physical parameters obtained during each batch

| BATCH | DISSOLVED OXYGEN (mgL ⁻¹) | pH | TEMPERATURE (°C) | SOLAR IRRADIANCE (uEm ⁻¹ s ⁻¹) |
|-------|---|-------------|---------------------|---|
| IC | 0.2 - 11.0 | 6.8 - 7.82 | 24 - 28 | 2400 |
| ICO | 0.3 - 14.0 | 6.9 - 7.82 | 24 - 28 | 2200 |
| IIC | 0.3 - 12.4 | 7.18 - 8.33 | 25 - 30 | 2000 |
| IICO | 0.3 - 14.2 | 7.16 - 8.41 | 25 - 30 | 2100 |
| IIIC | 0.03 - 10.5 | 7.76 - 8.4 | 25 - 32 | 2200 |
| IIICO | 0.03 - 18 | 7.75 - 8.4 | 25 - 32 | 2000 |
| IVM | 0.1 - 7.1 | 6.63 - 8.82 | 25 - 31 | 2800 |
| IVCO | 0.3 - 19.44 | 7.26 - 8.67 | 25 - 30 | 2400 |
| VC | 0.1 - 30.2 | 7.58 - 8.68 | 25 - 31 | 1600 |
| VMCO | 0.0 - 17.6 | 7.27 - 8.03 | 24 - 31 | 1600 |

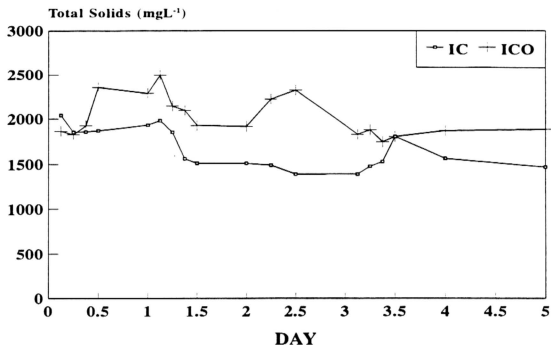


Fig 4.27: Total Solids changes in the HRAP treating RE (Batch I) where 5% CO₂ was bubbled in daily from 0630h to 1830h

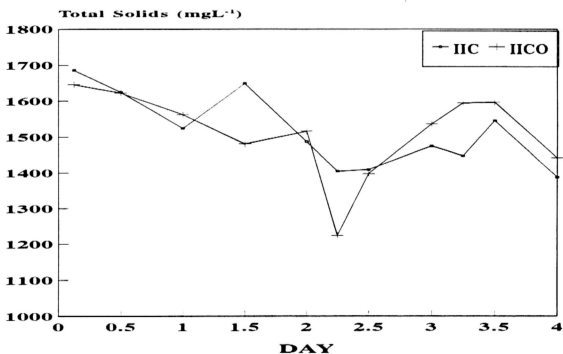


Fig 4.28: Total Solids changes in the HRAP treating RE (Batch II) where 5% CO₂ was bubbled in pond IICO from 0630h to 1830h

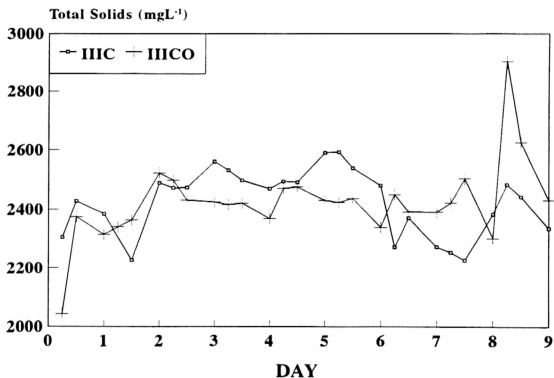


Fig 4.29: Total Solids changes in the HRAP treating RE (Batch III) where 5% CO₂ was bubbled in daily in IIICO from 0630h to 1830h

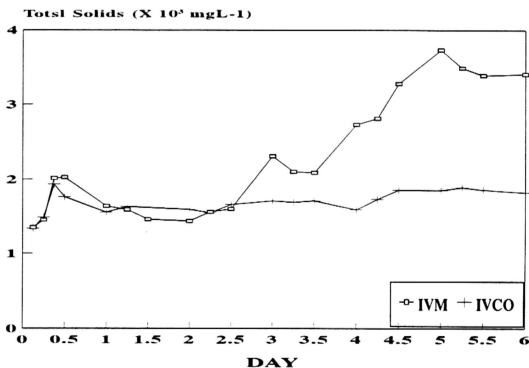


Fig 4.30: Total Solids changes in the HRAP treating RE (Batch IV), where 5% CO₂ was bubbled in daily from 0630h to 1830h IVCO and molasses was added in IVM at 1830h on day 2, 3 and 4

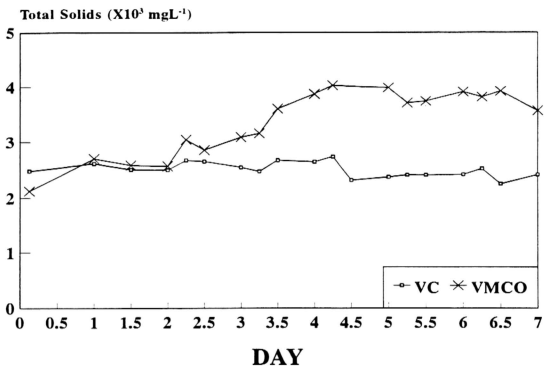


Fig 4.31: Total Solids changes in the HRAP treating RE (Batch V) where 5% CO_2 was bubbled in daily from 0630 to 1830h in VMCO and molasses was also added into VMCO at 1830h on day 2, 3 and 4

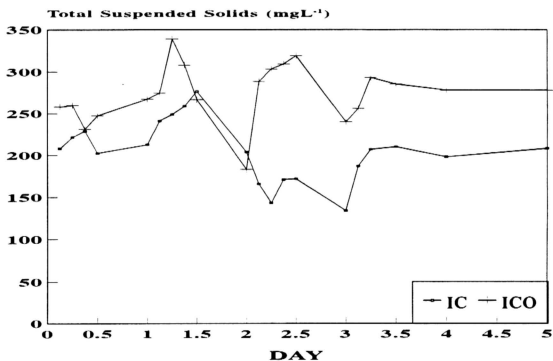


FIG 4.32: Total Suspended Solids in Batch I with CO_2 supplementation in ICO

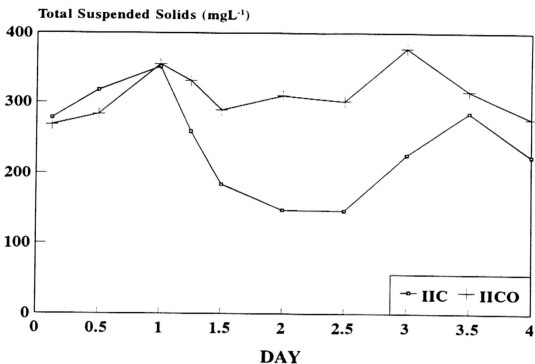


FIGURE 4.33: Total Suspended Solids of Batch II in the HRAP with CO_2 supplementation in IICO

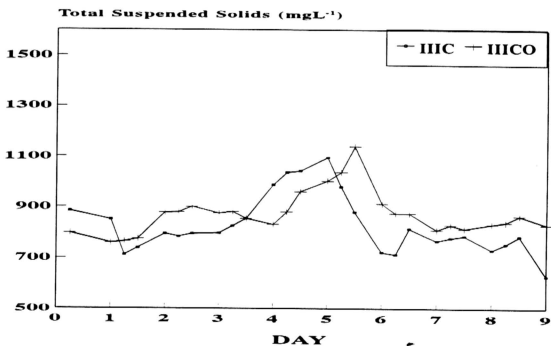


FIGURE 4.34: Total Suspended Solids in Batch III with CO_2 supplementation in the IICO

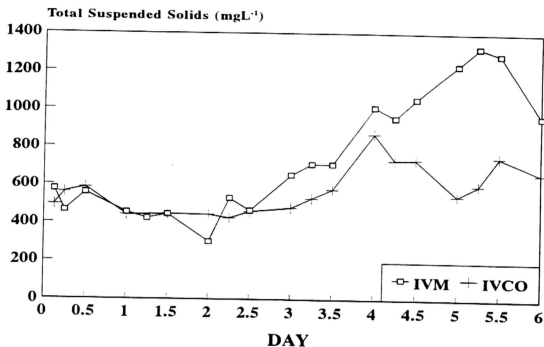


FIGURE 4.35: Total Suspended Solids in Batch IV in the HRAP with molasses supplemented in IVM and CO_2 bubbled in daily from 0630 to 1830h

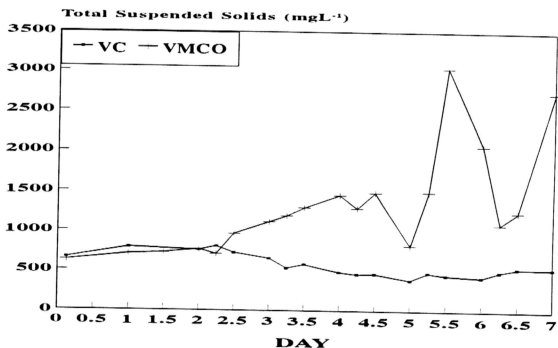


FIGURE 4.36: Total Suspended Solids in Batch V with CO_2 supplementation daily from 0630 to 1830 in VMCO and molasses at 1830h on day 2, 3 and 4 in VMCO too

4.6.1: Dissolved oxygen (DO) [Figure 4.37 to 4.41]

In Batches I, II and III, higher DO values were observed in the CO_2 ponds (14.0 to 18.0 mgL^{-1}) compared to the control ponds (10.5 to 12.4 mgL^{-1}) [Table 4.10]. Pond IVCO had a higher DO level (19.44 mgL^{-1}) than Pond IVM (7.1 mgL^{-1}). The lowest DO values observed were in pond VMCO and IVM where molasses was supplemented.

For all ponds, DO values were the highest between 1200h to 1600h when light intensity was highest. The highest DO value observed for the first three batches at midday corresponded to the start of the stationary phase of algal growth while for Ponds IVM and VMCO the highest DO was on the sixth day when molasses supplementation had been discontinued.

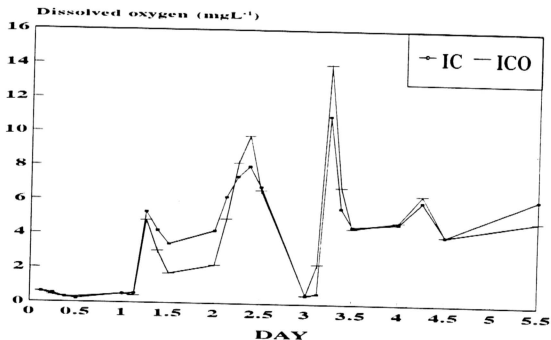


Fig 4.37: Dissolved oxygen levels in RE of HRAP (Batch I) where 5% CO_2 in air was bubbled in daily from 0630h to 1830h

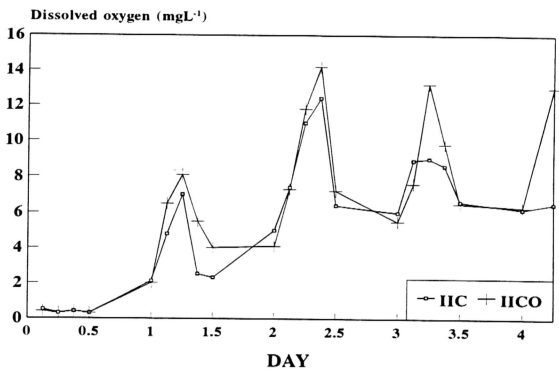


Fig 4.38: Dissolved oxygen levels in RE of HRAP (Batch II) where 5% CO_2 in air was bubbled in daily from 0630h to 1830h

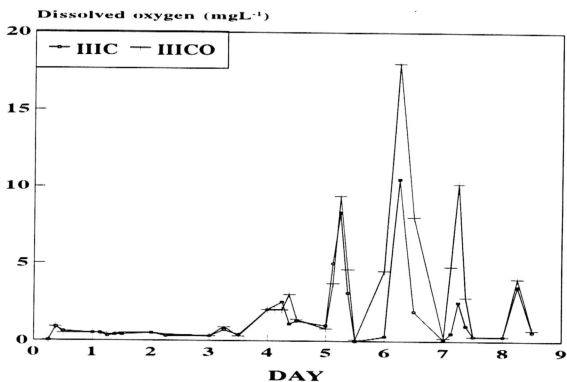


Fig 4.39: Dissolved oxygen levels in RE of HRAP (Batch III) where 5% CO_2 in air was bubbled in daily from 0630h to 1830h

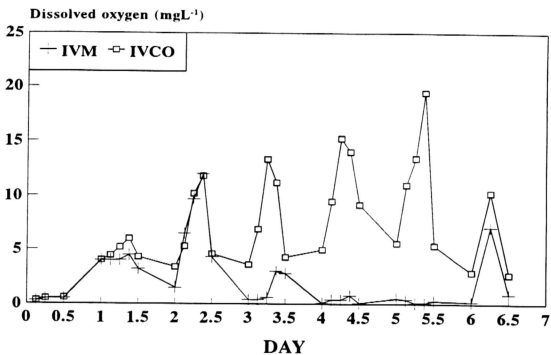


Fig 4.40: Dissolved oxygen levels of HRAP (Batch IV) where 5% CO_2 in air was bubbled daily from 0630 to 1830h in IVCO and molasses added into IVM on day 2.3 and 4 at 1830h

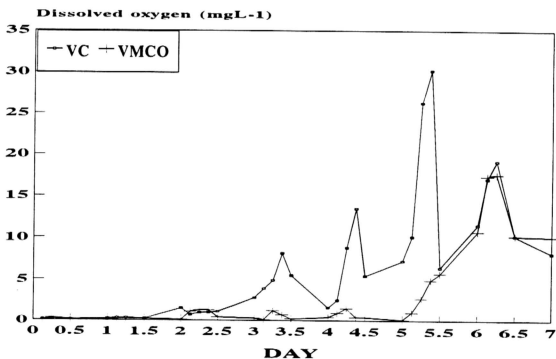


Fig 4.41: Dissolved oxygen levels in RE of HRAP (Batch V) where 5% CO_2 in air was bubbled in daily from 0630 to 1830 in VMCO and molasses was added in on day day 2,3 and 4 at 1830h in VMCO

4.6.2: pH (Figure 4.42 to Figure 4.46)

The pH of the ponds varied and was dependent on the time of day as well the algal growth phase. The pH peaked at midday for all batches. Highest pH were also obtained during stationary phase.

In ponds supplemented with CO_2 , pH was not lower than the control showing that active algal growth and photosynthesis utilized the CO_2 efficiently.

In Batches IV and V, pH decreased when molasses was added.

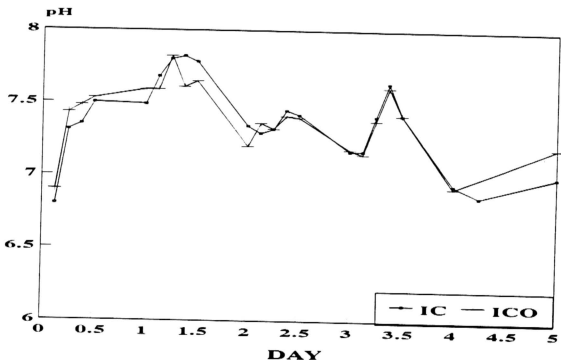


Fig 4.42: pH changes in HRAP (Batch I) where 5% CO_2 was bubbled in daily from 0630 to 1830h in ICO

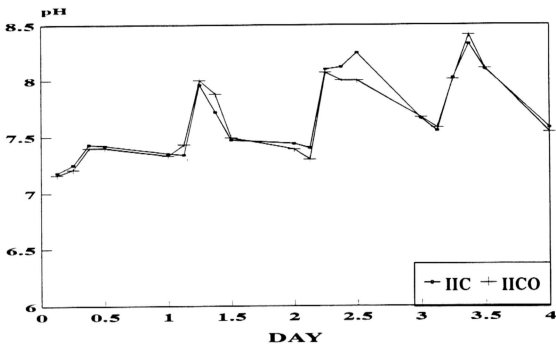


Fig 4.43: pH changes in the HRAP (Batch II) where 5% CO₂ was bubbled in daily from 0630 to 1830h in IICO

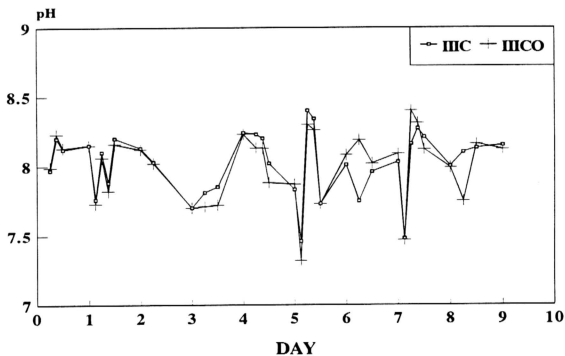


Fig 4.44: pH changes in the HRAP (Batch III) where 5% CO₂ was bubbled in daily from 0630 to 1830h in IICO

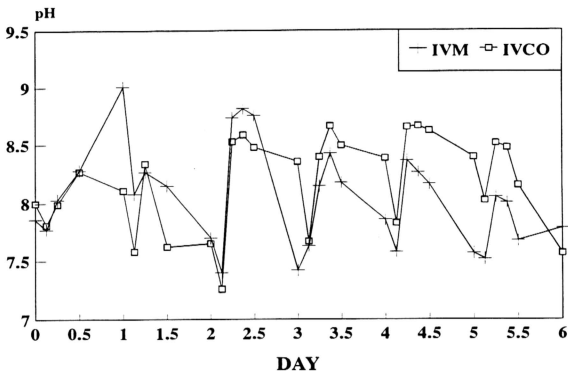


Fig 4.45: pH changes in HRAP (Batch IV) where 5% CO₂ was bubbled in daily from 0630 to 1830h in IVCO and 0.05% molasses was added on day 2,3 and 4 at 1830h into IVM

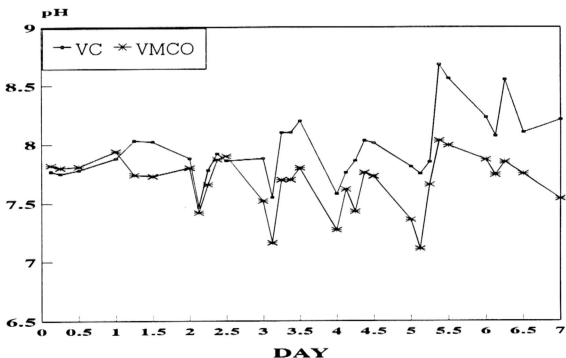


Fig 4.46: pH changes in the HRAP (Batch IV) where 5% CO₂ was bubbled in daily from 0630 to 1830h in VMCO and 0.05% molasses was added on day 2,3 and 4 at 1830h into VMCO too

4.6.3 Light irradiance and temperature

The daily changes in light irradiance and temperature are shown in Figures 4.47 to 4.56.

Light irradiance and temperature were highest during midday.

4.7: Biochemical composition of algal biomass

Biochemical composition of the algal biomass was determined to assess the suitability of the biomass for use as feed.

4.7.1: Biochemical composition in Batches I and II

Table 4.10 gives the biochemical composition of the algae from Batches I and II. CO₂ supplementation did not significantly change the biochemical composition.

4.7.2: Biochemical composition in Batches III to V

Figures 4.57 to 4.62 gives the biochemical composition of algae from Batches III to V on a daily basis. Highest lipid content was found in Pond IVCO (14.12%). Highest protein content was observed in Pond IVM (67.2%). There appears to be a general increase in carbohydrate content but decrease in lipid content as the cultures reached stationary phase.

Detailed values obtained for the biochemical composition of biomass is summarised in Appendix 12.

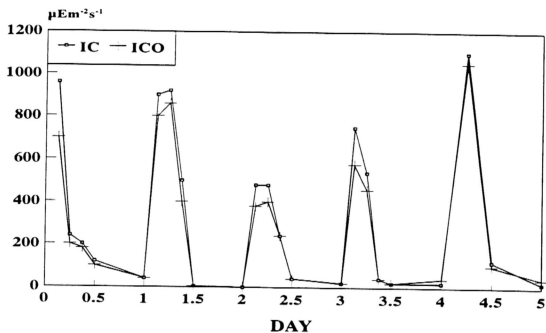


Fig 4.47: Irradiance surrounding the ponds during the HRAP study - Batch I

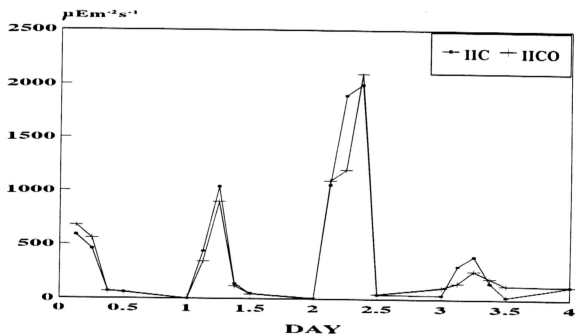


Fig 4.48: Irradiance surrounding the ponds during the HRAP study - Batch II

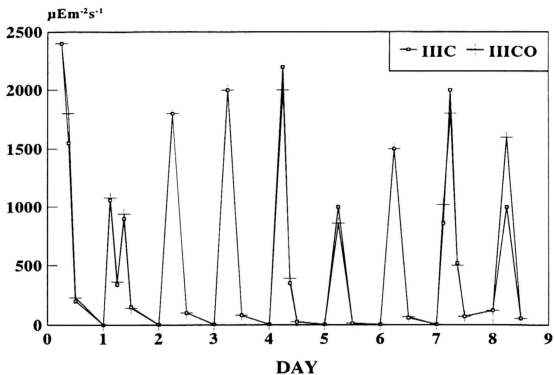


Fig 4.49: Irradiance surrounding the ponds during HRAP study - Batch III

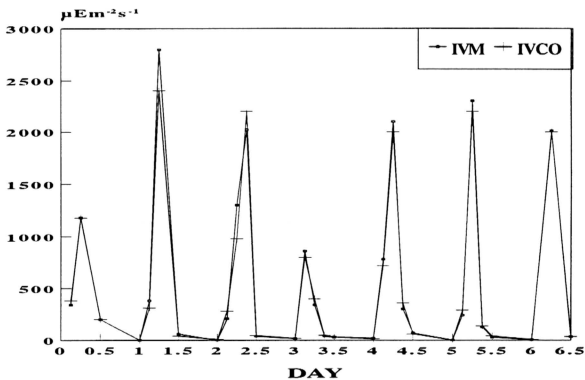


Fig 4.50: Irradiance surrounding the ponds during the HRAP study - Batch IV

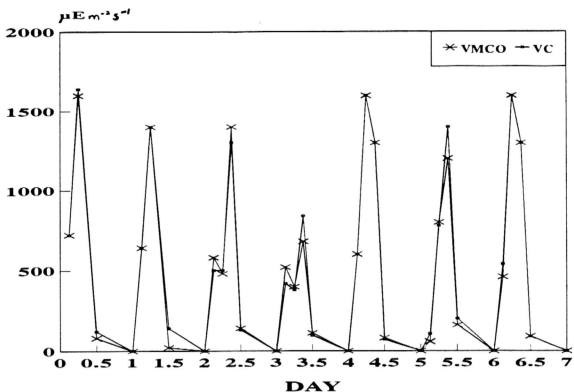


Fig 4.51: Irradiance surrounding the ponds during HRAP study - Batch V

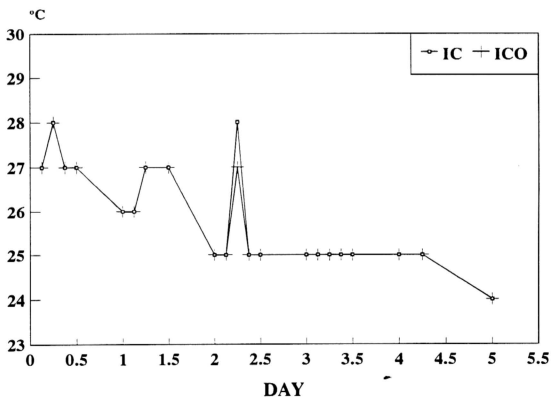


Fig 4.52: Temperature Profile of ponds during the HRAP study - BATCH I

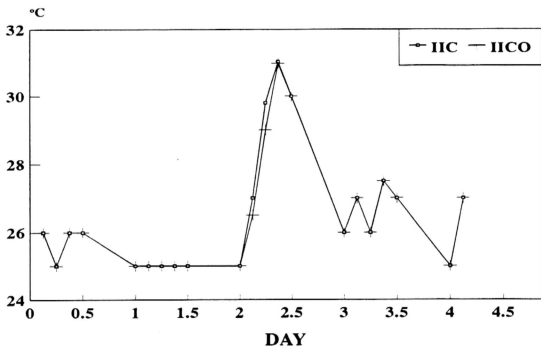


Fig 4.53: Temperature Profile during HRAP study in Batch II

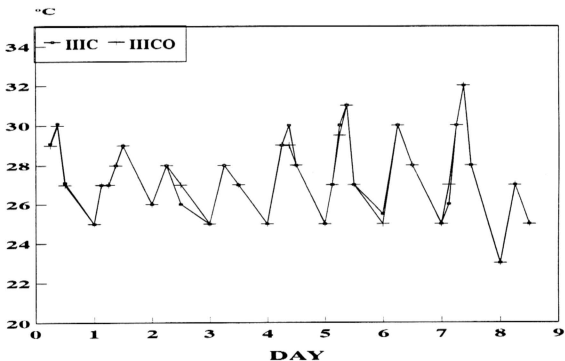


Fig 4.54: Temperature Profile of HRAP study - Batch III

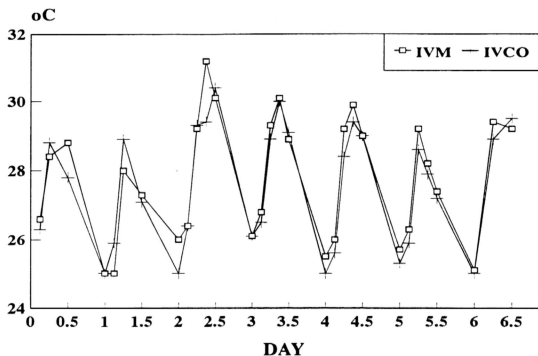


Fig 4.55: Temperature profile during HRAP study - Batch IV

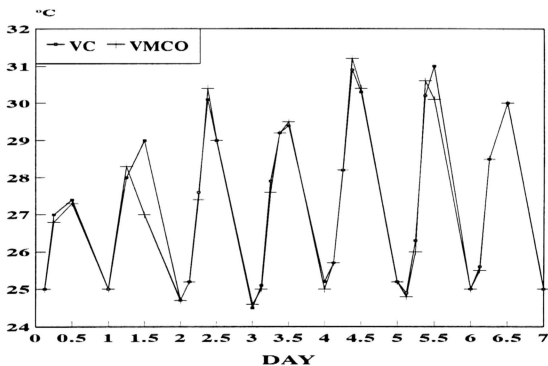


Fig 4.56: Temperature Profile during HRAP study - Batch V

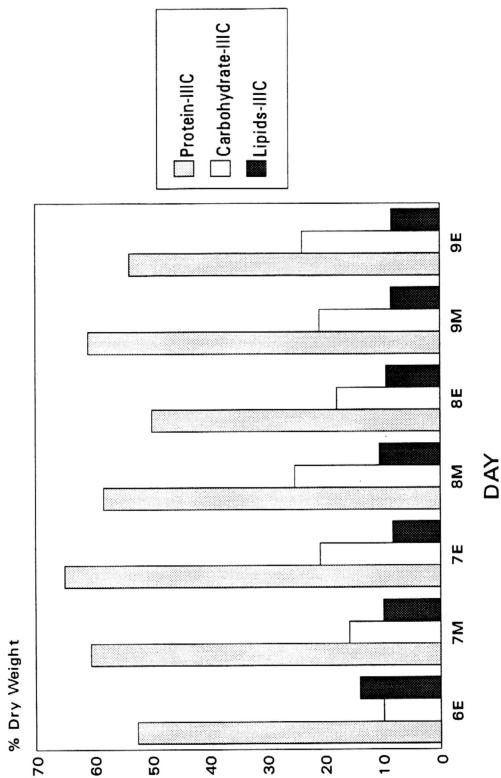


FIG 4.57 : Biochemical composition of algal biomass from Batch III - Control
(M = morning; E = evening)

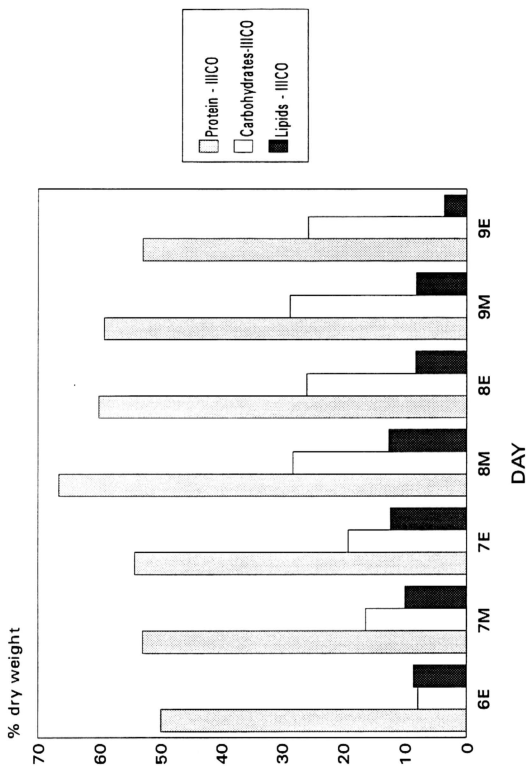


FIG 4.58 : Biochemical composition of algal biomass from Batch III - CO₂
(M = morning; E = evening)

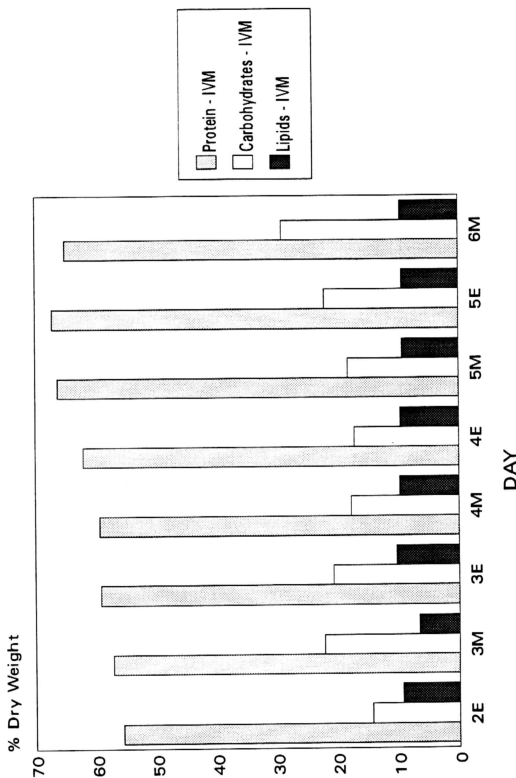


FIG 4.59 : Biochemical composition of algal biomass for Batch IV - Molasses
(M = morning; E = evening)

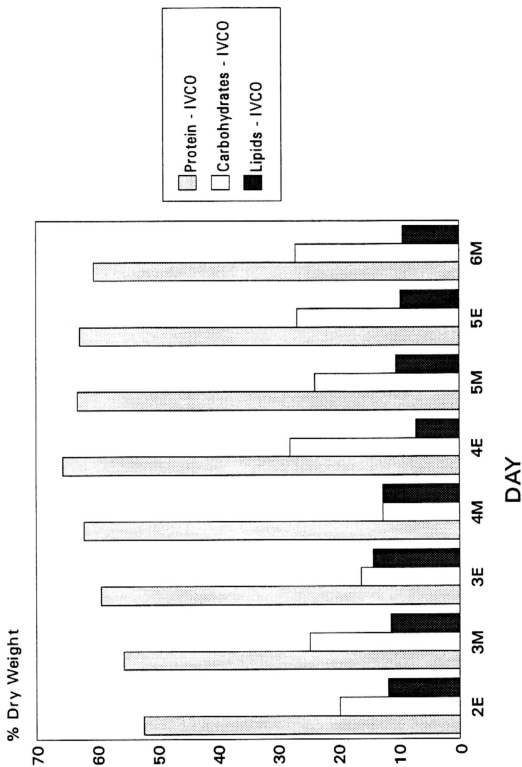


FIG 4.60 : Biochemical composition of algal biomass for Batch IV - CO₂
(M = morning; E = evening)

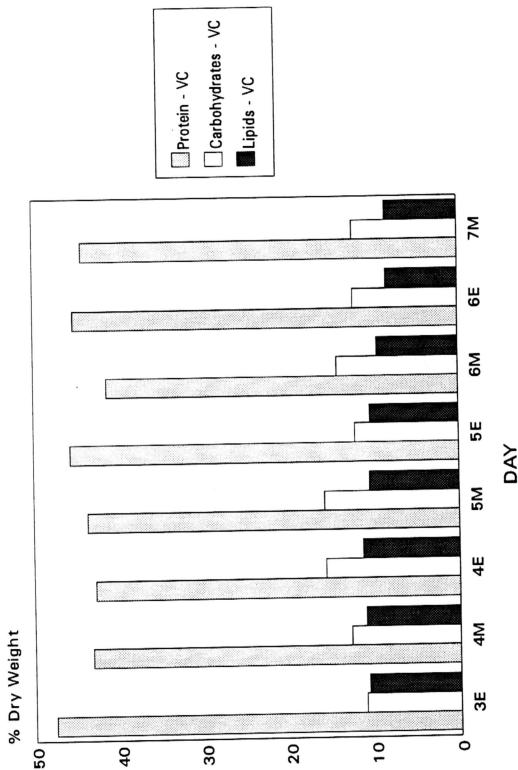


FIG 4.61 : Biochemical composition of algal biomass for Batch V - Control
(M = morning; E = evening)

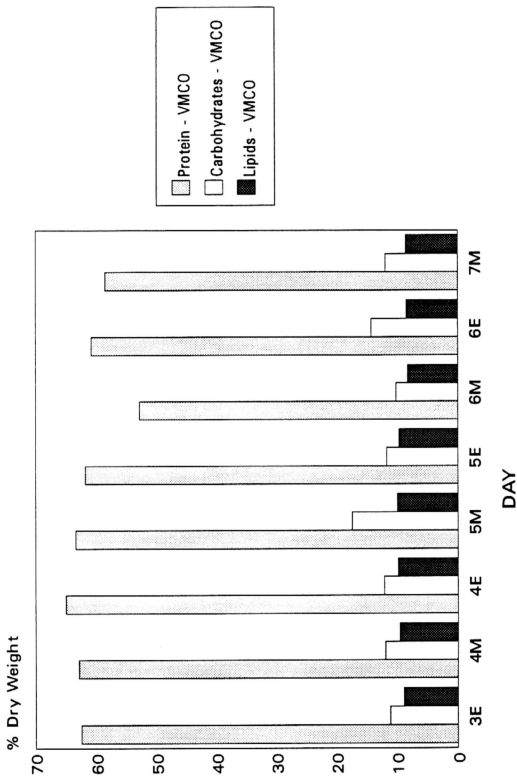


FIG 4.62 : Biochemical composition of algal biomass from Batch V - Molasses + CO₂
(M = morning; E = evening)

TABLE 4.10 : Biochemical composition of algal biomass obtained from HRAP – Batch I & II (Stationary phase)

| Batch | Protein | Carbohydrate | Lipids | Carotenoids |
|-------|---------|--------------|--------|-------------|
| IC | 56.3 | 8.4 | 10.2 | 0.6 |
| ICO | 60.2 | 12.5 | 11.6 | 0.8 |
| IIC | 58.3 | 12.5 | 9.65 | 0.6 |
| IICO | 61.9 | 15.6 | 13.3 | 0.7 |

(values are expressed in % DW & mg/g for carotenoid)

4.8: SEMIDIURNAL STUDIES

Altogether eight semidiurnal studies were carried out, one each for batch I and II and two each for batches III, IV and V. Studies were carried out during logarithmic and stationary phase of the growth cycle. Three studies were carried out during logarithmic phase, four during stationary phase and one in between logarithmic and stationary phase.

4.8.1: Algal Cell Density [Figure 4.63a to 4.63h]

In general, in the early morning hours, there was little to no increase in cell density. Increase in the cell density occurred in the morning from around 0730 to 0900h (Fig4.63a, 4.63b), or 1000h (Fig 4.63c, 4.63f). This was followed by a "stationary phase" with little increase in cell numbers. In the afternoon, a second

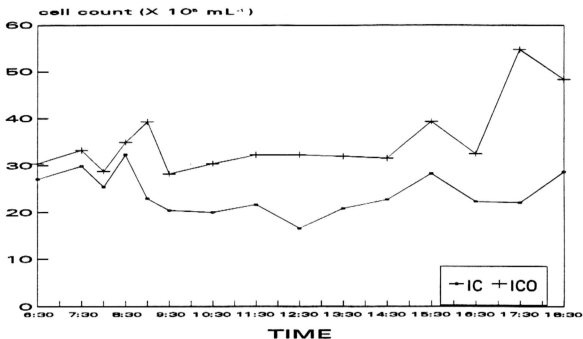


FIG 4.63 a : Semidiurnal changes in algal cell density in HRAP on day 2 Batch I

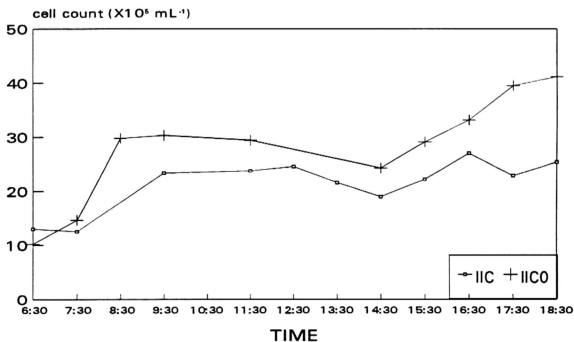


FIG 4.63b : Semidiurnal changes in algal cell density in HRAP on day 1 Batch II

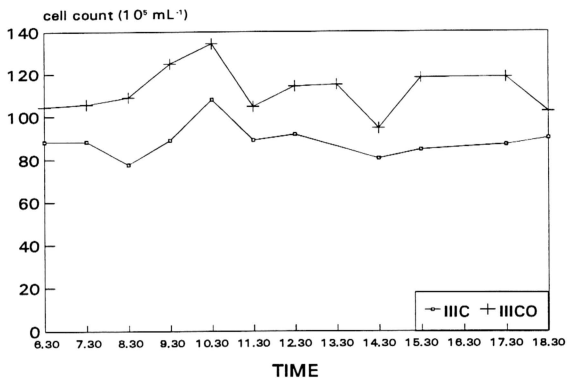


FIG 4.63c: Semidiurnal changes in algal cell density in HRAP on day 5 Batch III

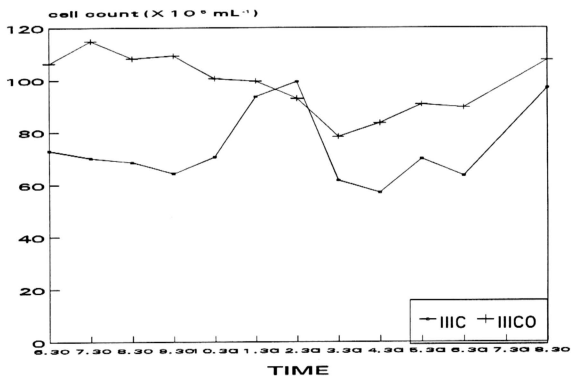


FIG 4.63 d : Semidiurnal changes in algal cell density in HRAP on day 7 Batch III

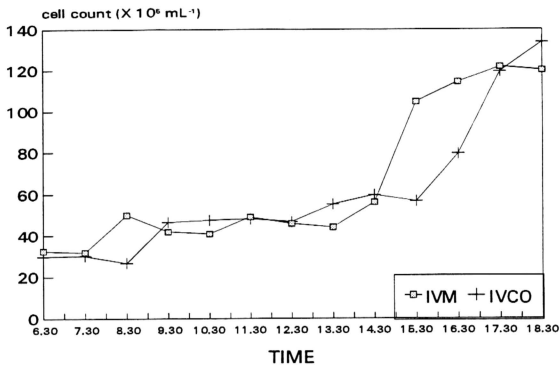


FIG 4.63 e : Semidiurnal algal density changes in the HRAP on day 2 Batch IV

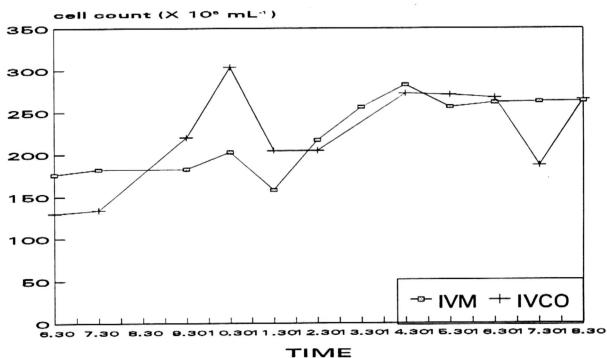


FIG 4.63 f : Semidiurnal algal cell density changes in the HRAP on day 4 Batch IV

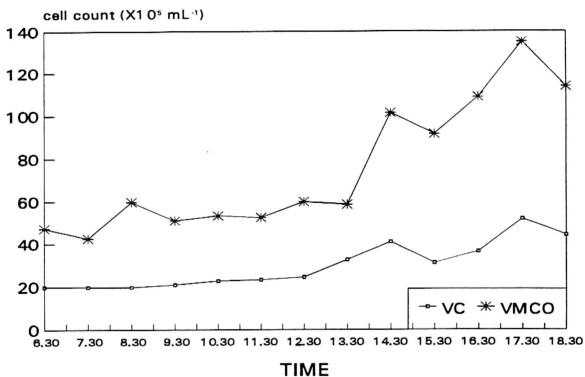


Fig 4.63g : Semidiurnal changes in algal cell density in HRAP on day 3 Batch V

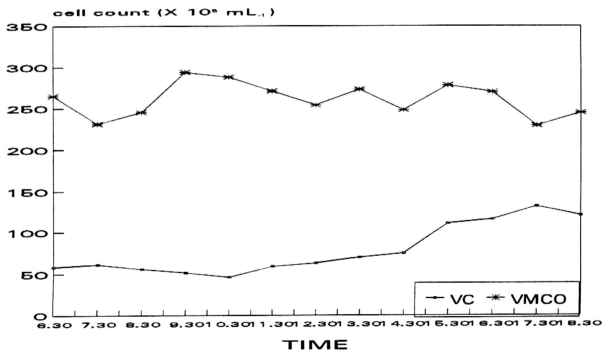


Fig 4.63h : Semidiurnal changes in algal cell density in HRAP on day 5 Batch V

period of increase in cell density occurred generally from 1430h to 1530h or 1630h (Fig4.63a, 4.63b, 4.63d, 4.63e & 4.63g).

Cell density increase during logarithmic phase was generally higher than during stationary phase. This is clearly shown by comparison of Figure 4.63e with 4.63f (Batch IV). Supplementation with CO_2 or CO_2 together with molasses resulted in increase cell density over the control.

4.8.2: Chlorophyll-a content [Figure 4.64a to 4.64h]

Semidiurnal changes in chl-a content generally followed changes in the algal cell density. During logarithmic phase, chl-a increased from early morning till late evening as a result of increase in cell number (growth). During stationary phase chl content was generally high during bright sunshine hours (Figure 4.64d, 4.64f, 4.64h).

4.8.3: Chl-a content per cell (Figure 4.65a to 4.65h)

In general there was an increase in chl-a per cell in the morning, being highest during midday and high sunshine hours (Figure 4.65a, 4.65b, 4.65d, 4.65e). There is a general decline towards the evening (Figure 4.65a, 4.65d, 4.65e, 4.65f, 4.65g and 4.65h).

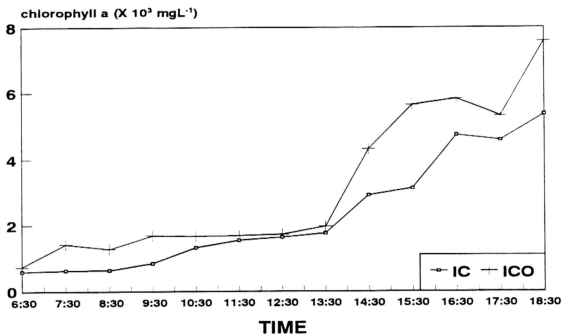


Fig 4.64a: Semidiurnal changes in chlorophyll a concentration in HRAP on day 2 Batch I

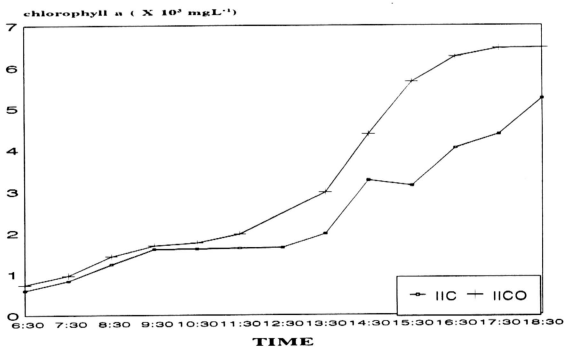


FIG 4.64 b : Semidiurnal changes in chlorophyll a concentration in the HRAP on day 1 Batch II

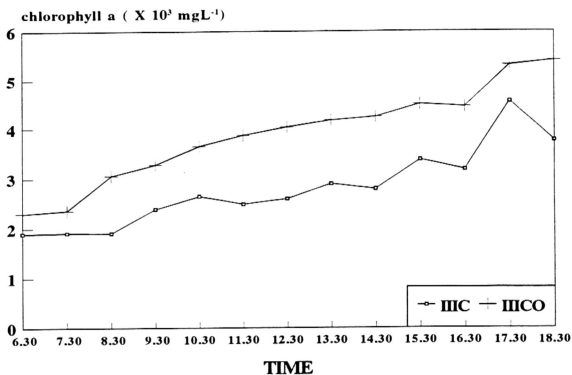


FIG 4.64c : Semidiurnal changes in chlorophyll a concentration in the HRAP on day 5 Batch III

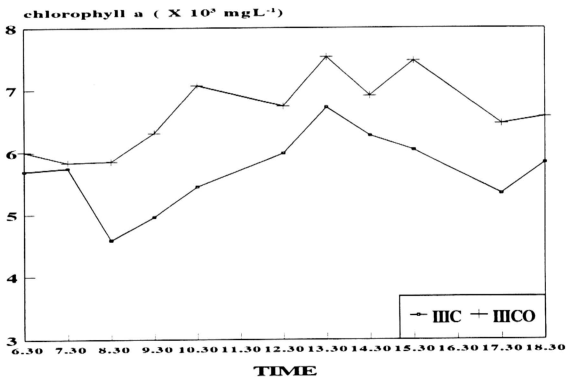


FIG 4.64 d : Semidiurnal changes in chlorophyll a concentration in the HRAP on day 7 Batch III

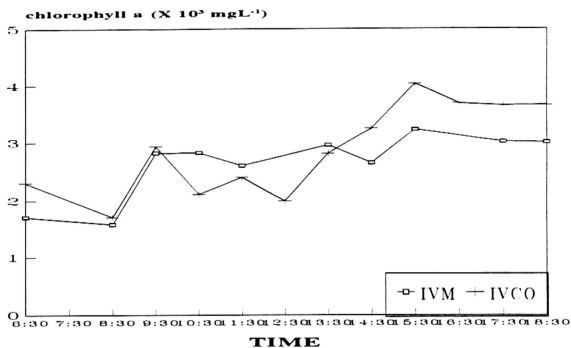


FIG 4.64 e : Semidiurnal changes in chlorophyll concentration in the HRAP on day 2 Batch IV

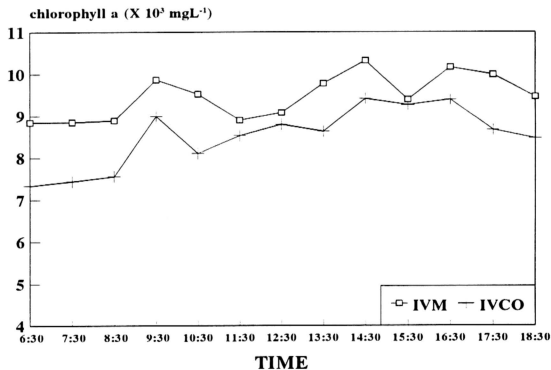


FIG 4.64 f : Semidiurnal changes in chlorophyll a concentration in a HRAP on day 4 Batch IV

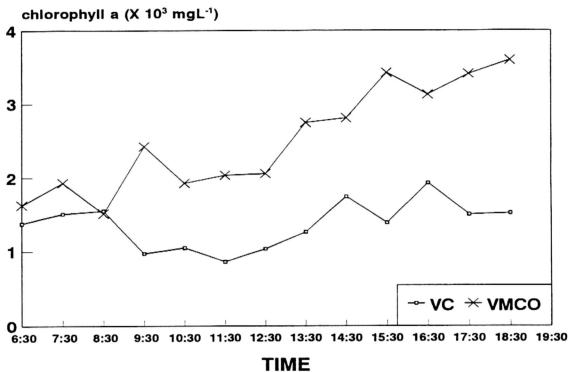


FIG 4.64 g : Semidiurnal changes in chlorophyll a concentration in HRAP on day 3 Batch V

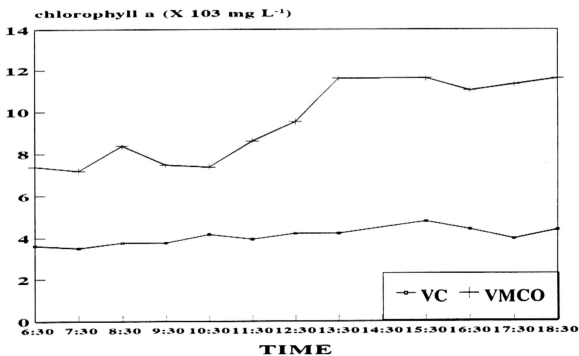


FIG 4.54 h : Semidiurnal changes in chlorophyll a concentration in HRAP on day 5 Batch V

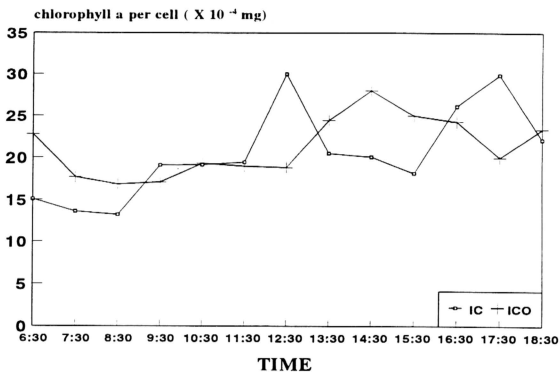


FIG 4.65 a : Semidiurnal changes in chlorophyll a per cell in the HRAP on day 2 Batch I

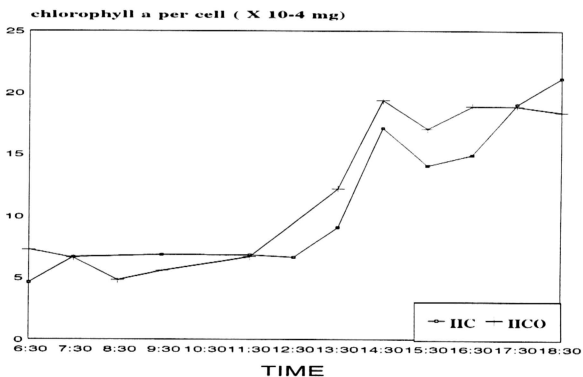


FIG 4.65 b : Semidiurnal changes in chlorophyll a per cell in the HRAP on day 1 Batch II

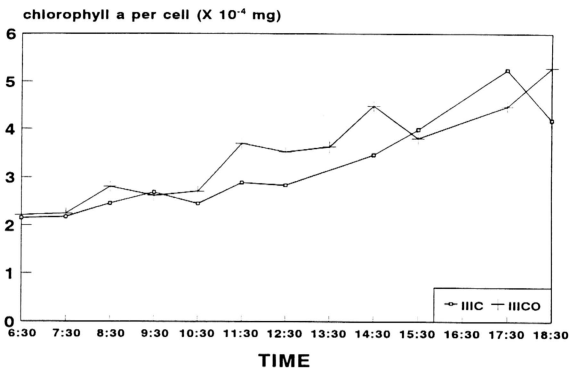


FIG 4.65 c : Semidiurnal changes in chlorophyll a per cell in the HRAP on day 5 Batch III

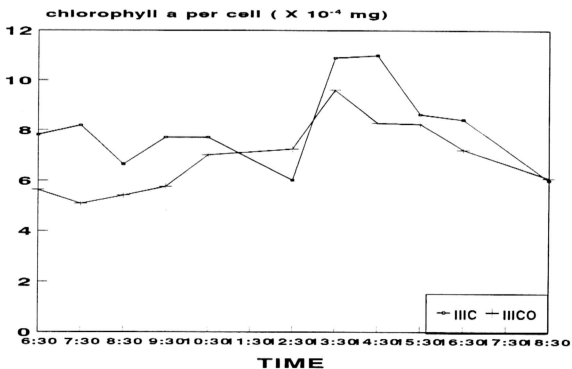


FIG 4.65 d : Semidiurnal changes in chlorophyll a per cell in the HRAP on day 7 Batch III

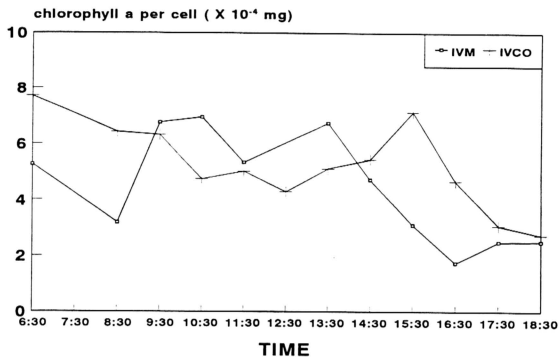


FIG 4.65 e : Semidiurnal changes in chlorophyll a per cell in the HRAP on day 2 Batch IV

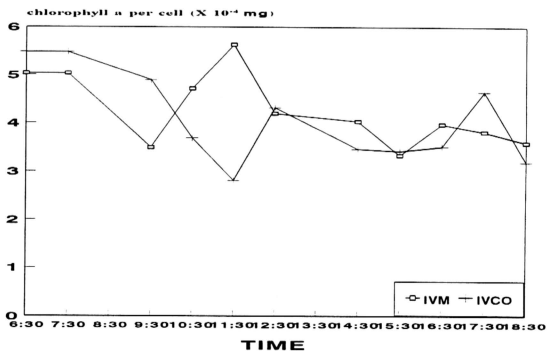


FIG 4.65 f : Semidiurnal changes in chlorophyll a per cell in the HRAP on day 4 Batch IV

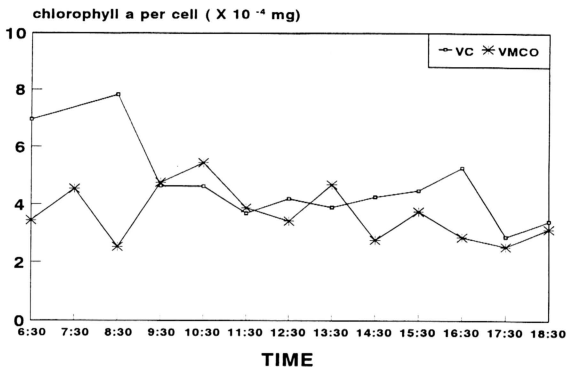


FIG 4.65 g : Semidiurnal changes in chlorophyll a per cell in the HRAP on day 3 Batch V

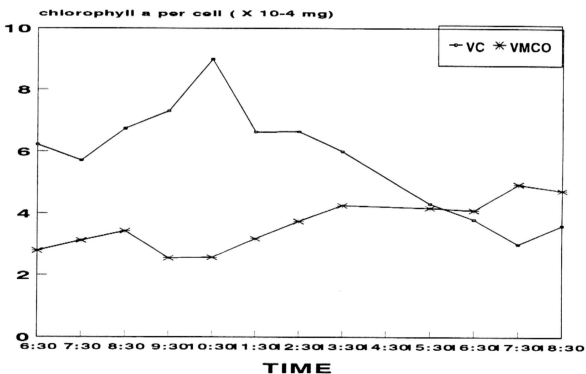


FIG 4.65 h : Semidiurnal changes in chlorophyll a per cell in the HRAP on day 5 Batch V

4.8.4: Dissolved Oxygen (Figures 4.66a to 4.66h)

DO levels indicate photosynthesis and cell growth. In general, through the day DO levels increase with increasing light intensity after an early morning lag phase. A peak in DO levels was observed around midday, after which DO declined slightly towards evening. Addition of molasses resulted in lower DO levels than the control. (Figure 4.66f, 4.66g and 4.66h)

4.8.5: pH levels [Figure 4.67a to 4.67h]

pH also indicates photosynthesis when inorganic carbon is utilized. In general the pH changes throughout the time monitored also followed cell growth and photosynthesis. High pH levels were observed in the afternoon. On few occasions a drop in light intensity during the day resulted in a corresponding dip in the pH (Figure 4.67a and 4.67g).

In general pH levels in all occasions fell within the range of 7.14 to 8.8 which is suitable for algal growth.

4.8.6: Irradiance and Temperature conditions

Semidiurnal changes in irradiance and temperature are shown in Figures 4.68a to 4.68h and 4.69a to 4.69h respectively. Abrupt changes in light levels are due to cloud cover at the point when measurement was taken.

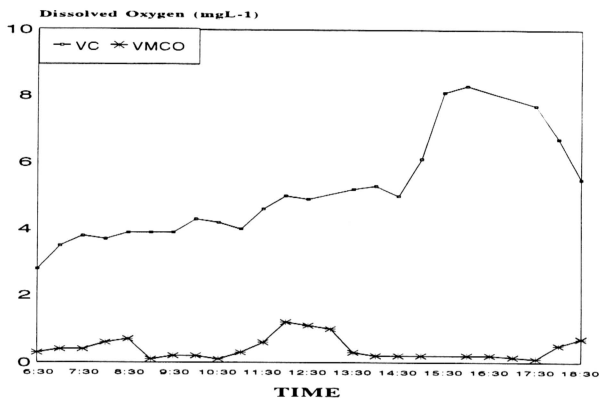


FIG 4.66 g : Semidiurnal changes in dissolved oxygen in the HRAP on day 3 Batch V

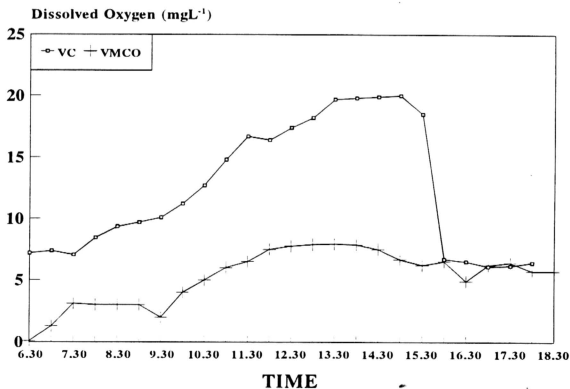


FIG 4.66 h : Semidiurnal changes in dissolved oxygen in the HRAP on day 5 Batch V

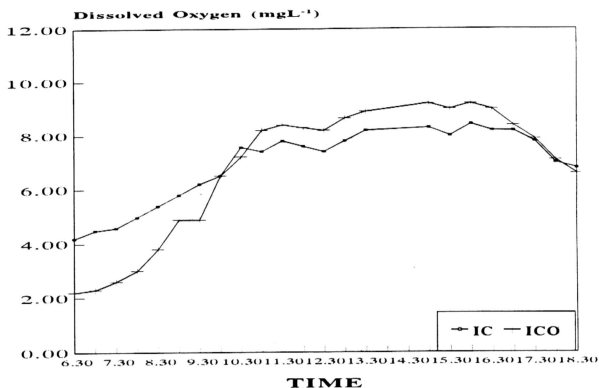


FIG 4.66a : Semidiurnal changes in dissolved oxygen in the HRAP on day 2 Batch I

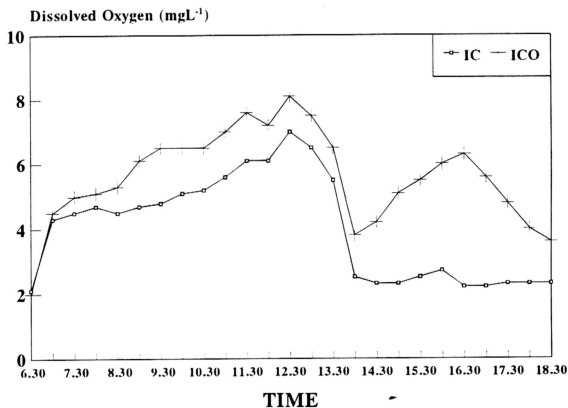


FIG 4.66 b : Semidiurnal changes in dissolved oxygen in the HRAP day 1 Batch II

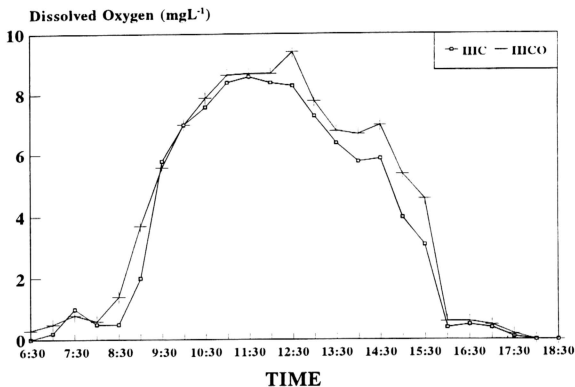


FIG 4.66 c : Semidiurnal changes in dissolved oxygen in the HRAP on day 5 Batch III

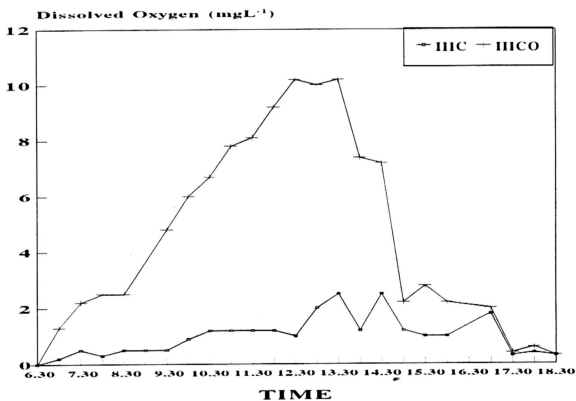


FIG 4.66d : Semidiurnal changes in dissolved oxygen in the HRAP on day 7 Batch III

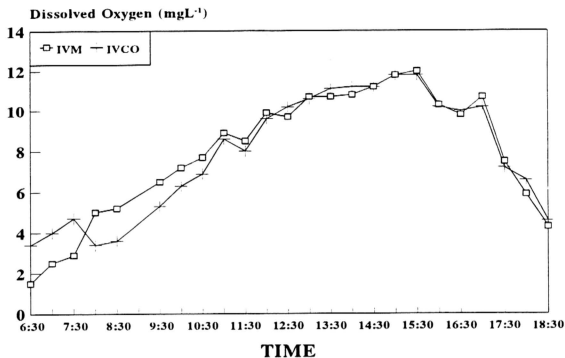


FIG 4.66 e : Semidiurnal changes in dissolved oxygen in the HRAP on day 2 Batch IV

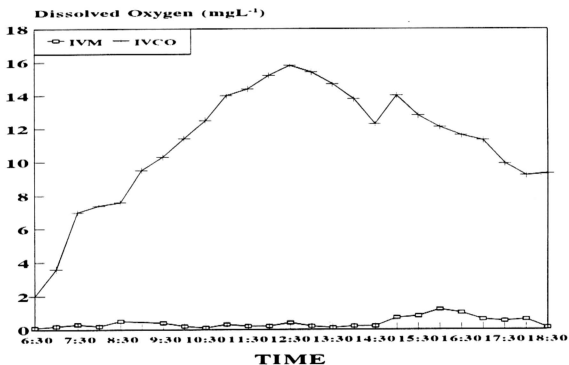


FIG 4.66 f : Semidiurnal changes in dissolved oxygen in the HRAP on day 4 Batch IV

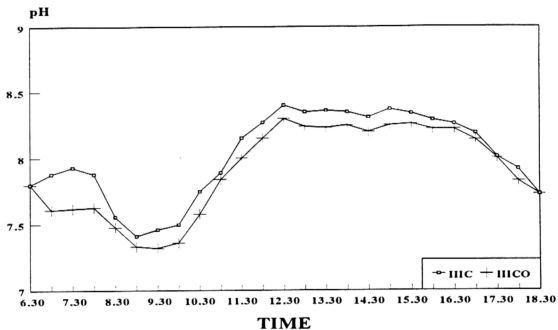


FIG 4.67 c : Semidiurnal changes in pH in the HRAP on day 5 Batch III

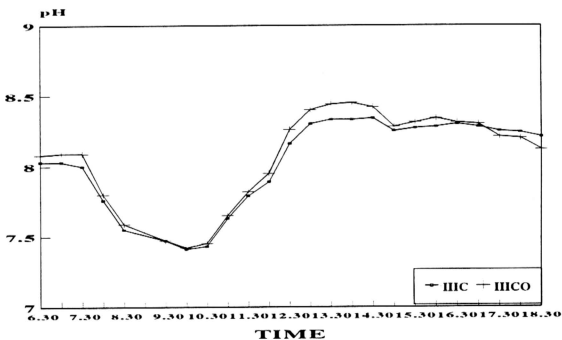


FIG 4.67 d : Semidiurnal changes in pH in the HRAP on day 7 Batch III

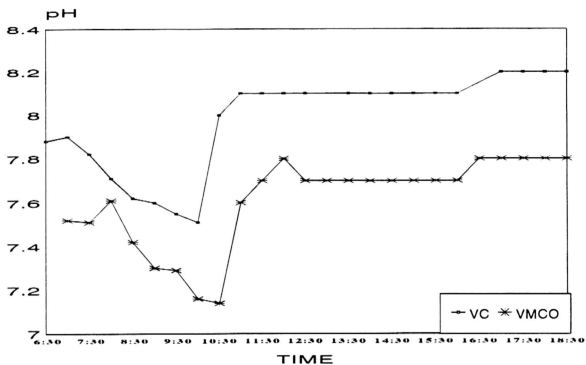


FIG 4.67 g : Semidiurnal changes in pH in the HRAP on day 3 Batch V

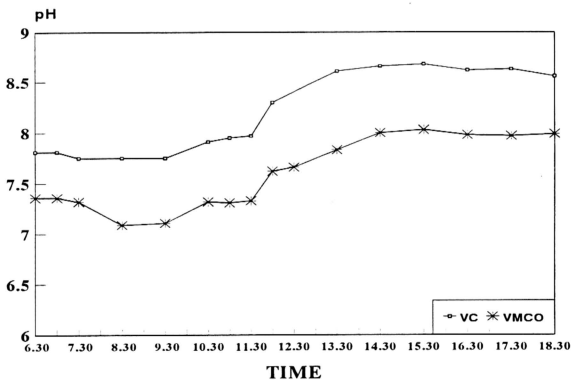


FIG 4.67 h : Semidiurnal changes in pH in the HRAP on day 5 Batch V

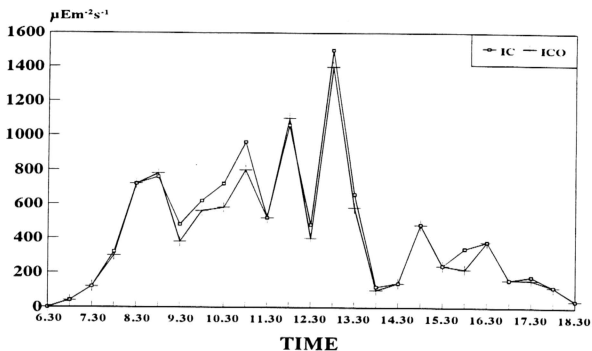


FIG 4.68 a : Semidiurnal changes in solar irradiance in the HRAP on day 2
Batch I

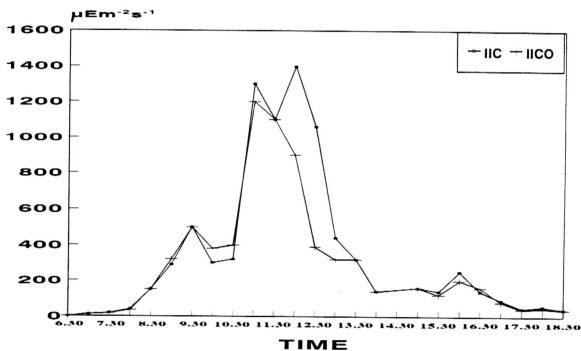


FIG 4.68 b : Semidiurnal changes in solar irradiance in the HRAP on day 1
Batch II

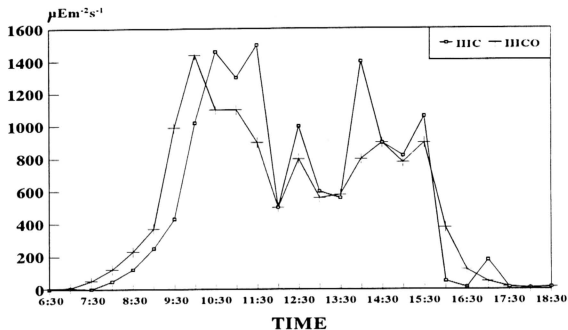


FIG 4.68 c : Semidiurnal changes in solar irradiance in the HRAP on day 5
Batch III

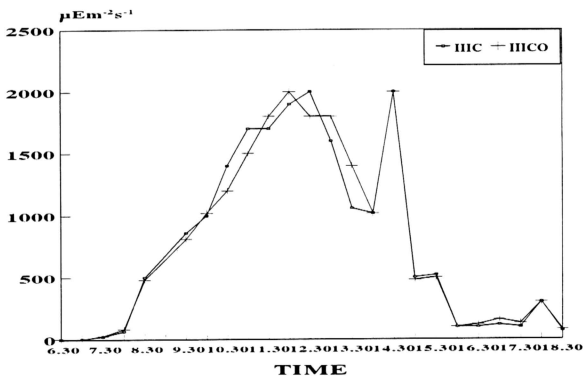


FIG 4.68 d : Semidiurnal changes in solar irradiance in the HRAP on day 7
Batch III

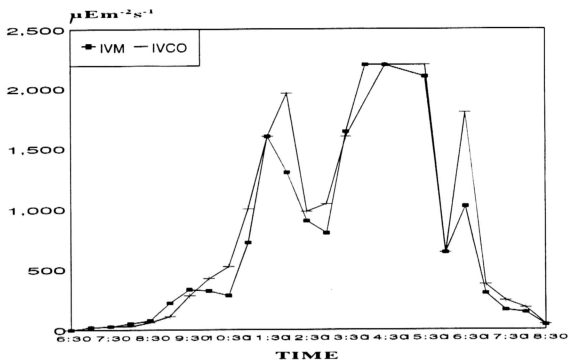


FIG 4.68 e : Semidiurnal changes in solar irradiance in the HRAP on day 2 Batch IV

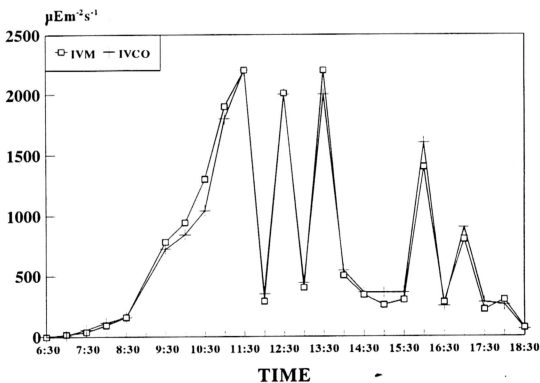


FIG 4.68 f : Semidiurnal changes in solar irradiance in the HRAP on day 4 Batch IV

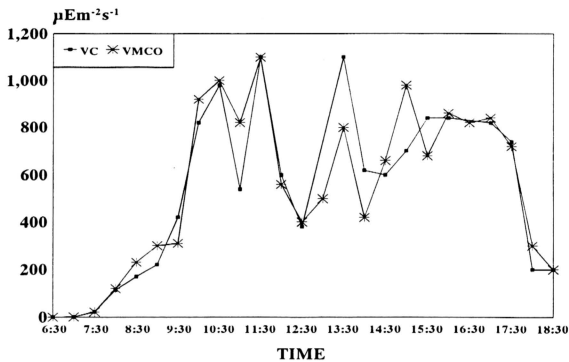


FIG 4.68 g : Semidiurnal changes in solar irradiance in the HRAP on day 3 Batch V

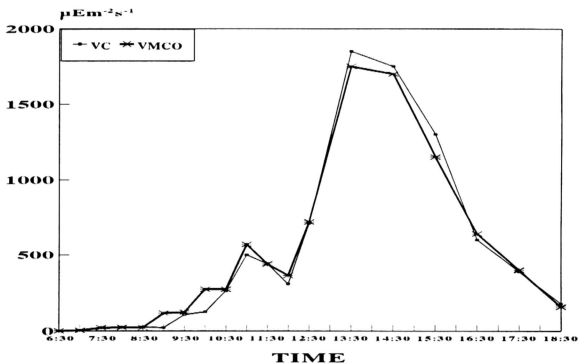


FIG 4.68 h : Semidiurnal changes in solar irradiance in the HRAP on day 5 Batch V

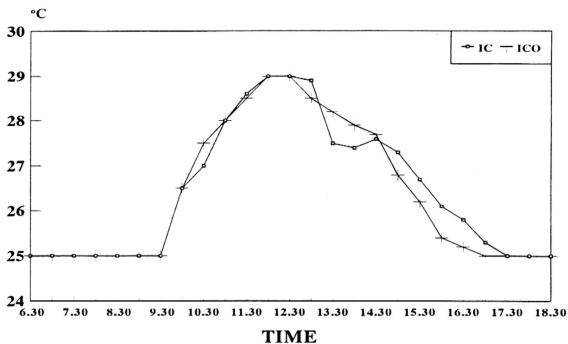


FIG 4.69 a : Semidiurnal changes in temperature in the HRAP on day 2
Batch I

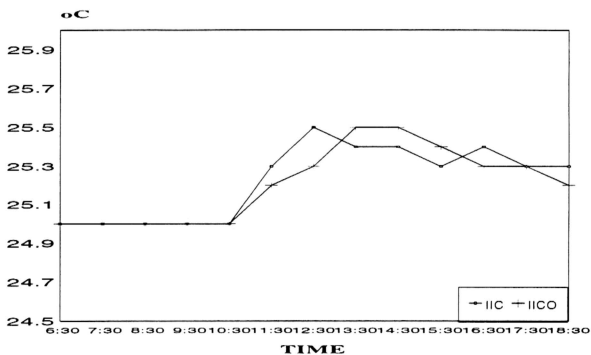


FIG 4.69 b : Semidiurnal changes in temperature in the HRAP on day 1
Batch II

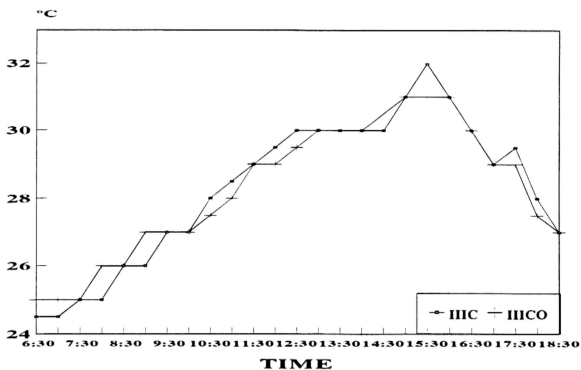


FIG 4.69 c : Semidiurnal changes in temperature in the HRAP on day 5
Batch III

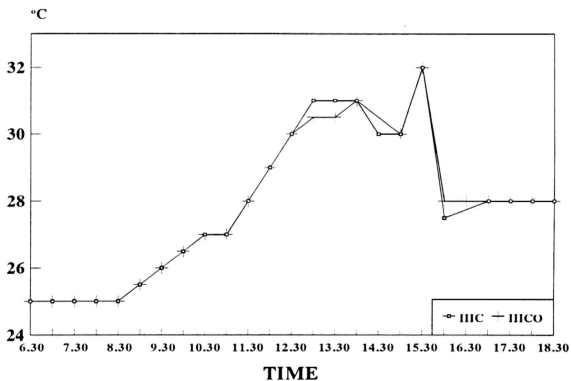


FIG 4.69 d : Semidiurnal changes in temperature in the HRAP on day 7
Batch III

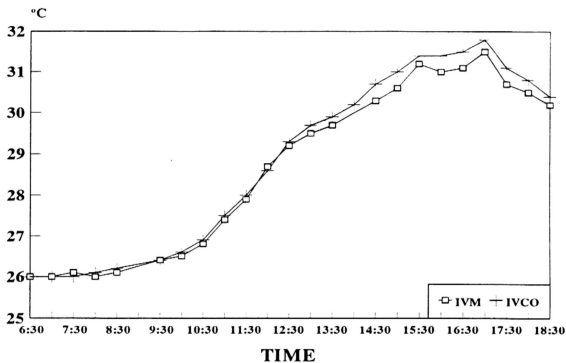


FIG 4.69 e : Semidiurnal changes in temperature in the HRAP on day 2
Batch IV

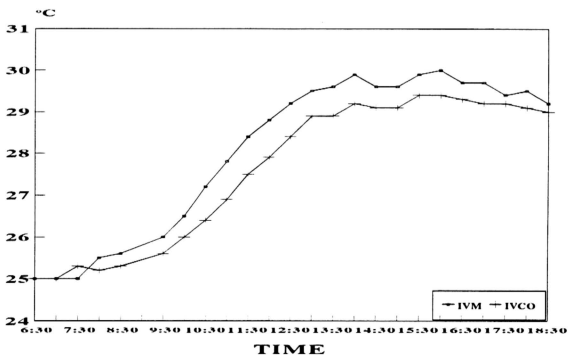


FIG 4.69 f : Semidiurnal changes in temperature in the HRAP on day 4
Batch IV

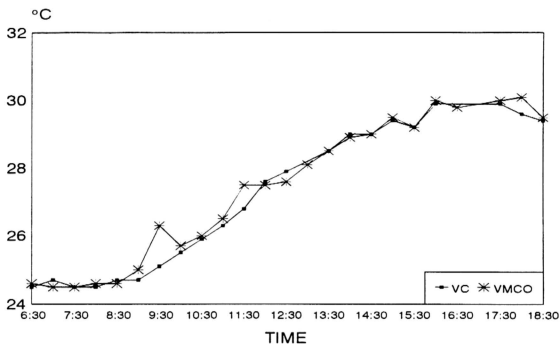


FIG 4.69 g : Semidiurnal changes in temperature in the HRAP on day 3
Batch V

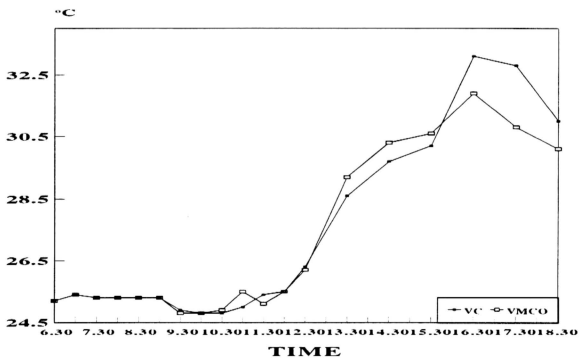


FIG 4.69 h : Semidiurnal changes in temperature in the HRAP on day 5
Batch V